Addis Abeba University

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School of Chemical and Bio-Engineering

Extraction and Evaluation of Fixed Oil from Black Cumin (Nigella Sativa) using Hexane and Ethanol Solvents

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This is to certify that thesis is prepared by Shimeles Mekonnen, entitled: **Extraction and Evaluation of Fixed Oil from Black Cumin (Nigella Sativa) using Hexane and Ethanol Solvents** and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Process Engineering complies with the regulations of the University and meets the accepted standards with respect to Originality and quality

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DECLARATION

I declare that this thesis for the M.Sc. Degree at Addis Ababa University, hereby submitted by me, is my original work and has not previously been submitted for the degree at this or any other university, and that all resources of materials used in this thesis have been duly acknowledged.

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Abstract

Black cumin seed (Nigella sativa) oil was used traditionally in different countries for many medical complains. The seeds are used for culinary purposes, medicinal treatment and also used as food additives. The oil can produce by different extraction methods. Among of those methods, solvent extraction is the most promising and effective process with minimum cost. When we are using solvent extraction process, the major problem is the uses of toxic chemical that is hexane as a solvent. These solvent produces photochemical oxidants in the ozone by reacting with other pollutants during extraction and recovery process and also a trace of hexane that is found in the oil affects the human health. This study mainly investigates and compares the effect of solvents, ethanol and hexane in the extraction process and optimization was done in the two factors named as, extraction time and seed to solvent ratio. The extraction process was carried out by using 3 level factor of the seed to solvent ratio (1:4, 1:6 and 1:8) and 4 level of extraction time (2hr, 4hr, 6hr and 8hr) using for each solvents using soxhlet extractor. The maximum yield of extraction for hexane solvent was found to be 35.725% with seed solvent ratio 1:8 and 4 hour and the minimum yield were 20.575% with the seed solvent ratio 1:4 and 2 hour of extraction time. Whereas using ethanol solvent the higher extraction yield was 35.51% with the factor of the 1:6 of seed solvent ratio and with extraction time 6 hour and the minimum result were 22.81%withthe seed solvent ratio 1:4 and 2 hours. The significance of each parameter was checked by using design expert software with the p-value<0.05 and identify the components and functional group of the samples by using GC-MS and FTIR and identified 23 components and 10 functional groups. The physical properties, density, specific gravity, viscosity and pH; the chemical properties such as Acid value, saponification value and Iodine value of the fixed oil was done before refining and after refined. Both of the experiment results has almost the same components and functional groups and there is no any significance effect in the environment and for the human health in ethanol solvent extraction. Therefore it is better to use ethanol as a solvent for extraction process rather than hexane.

Key Words: Black cumin seed, HSO, ESO, solvent extraction, GC-MS, FT-IR.

List of Acronyms

A.O.A.C	Association of Official Analytical Chemists
A.V	Acid Value
ANOVA	Analysis of Variance
ESO	Ethanol Solvent Oil
FFA	Free Fatty Acid
FTIR	Fourier Transform Infrared
GC-MS	Gas Chromatography Mass Spectrometer
GRAS	generally Recognized AS Safe
HAP	Hazardous Air Polutant
HDLs	high density lipoproteins
HSO	Hexane Solvent Oil
I.S.I	Indian Standard Institute
I.V	Iodine Value
LDLs	Low Density Lipoprotein
S.V	Saponification Value

1. INTRODUCTION

1.1. Background

For centuries, in most of the ethnic community throughout the world, all kinds of plants have been used as different medicines. The potential source of natural antioxidants is plants, fruits and vegetables (Walton NJ, Brown DE, 1999).The belief of humans on plants to cure various diseases is as old as their history and developments in the domain of nutrition unveiled therapeutic potential of many culinary herbs during last few decades' (Hameed *et al*., 2008; Jabeen *et al*., 2008).Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of human kind. The search for eternal health and longevity and for remedies to relieve pain and discomfort drove prehistoric man to use many plants. Currently the separation of extracts from natural sources especially from different plants is an activity of great interest, because of they have different chemicals compounds that have a potential applications in cosmetics, food and pharmaceutical industry (Brunner 2003). A lot of work has been carried out on analysis of seed oils by a number of workers, primarily because of extensive demands for oils both for human consumption and for industrial applications; consequently there is an increasing need to search for oils from non-conventional sources to augment the available ones and also to meet specific applications (M.Z. Kyari, 2007).

Plant-derived oils can be broadly classified into two, Essential Oils & Fixed Oils. Essential oils are volatile, and are usually derived from the non-seed parts of the plants. Essential oils have been used for centuries. They have been used extensively in ancient Rome, Greece, Egypt & the Middle East – as perfumes, flavors, deodorants, antiseptics & pharmaceuticals. As a result of new processing technologies, they can today used for more functions as well. Fixed oils are oils obtained from plants that are fatty, dense and non-volatile, are derived from seeds, nuts & vegetables. The use of fixed oils or "fatty oils" are more recent, but they have become as important as, or more important than, their essential oil counterparts, as edible oils, as industrial raw materials and feedstock for producing a number of useful products. The fixed oil is conventionally extracted by mechanical cold-pressing process or using a solvent extraction. Conventional methods such as solvent extraction and soxhlet, although effective for extraction, can lead to degradation of heat sensitive compounds as well as leave traces of toxic solvents in the solute. This is a concern for food and pharmacy industry, because of the increasing regulation of harmful solvents used (Aguilera *et al*., 2011).

Various methods of extraction employed have their own degrees of limitations, but all of them have common objectives such as to obtain the oil in its native form, the final yield of the oil should be maximum ,and to produce the residual oil cakes in such a form that the proteins and other non-lipids have the greatest possible value.

Black cumin is an annual herbaceous plant belonging to the Ranunculaceae family (Atta, M.B. 2003). It tastes slightly bitter and peppery with a crunchy texture. Black Cumin is also known as Black Seed, Black Caraway Seed, Habba Sawda (the Black Seed), Habbatul Baraka (the Blessed Seed), and by its botanical name "Nigella Sativa. And also named Roman coriander, nigella, originates from south and southwest Asia and has been cultivated in the tropics, subtropics and temperate regions like central Europe on a large scale. Depending on the way and the region of cultivation Nigella seeds contain more than hundred different volatile components (Khan, 1999, Paarakh, 2010). The seeds are angular, of generally small size (1-5 mg), dark grey or black color has length about 0.3 cm each. The seeds contain fixed and essential oils, alkaloids, proteins and saponin. Most of the biological activity has been attributed to the major components of the essential oil – thymoquinone– (24.5-57%), ρ-cymene (10.7-40.3%), a-thujen (1.9-8.2%), carvacrol (2,2-4.5%), 4-terpineol (1.9-4.5%) (Khan, 1999; Burits, 2000; El-Ghorab, 2003).

Black cumin (Nigella sativa L.) is one of the most medicinal seeds in history. Even if black cumin seed is mentioned in religious books, it was not carefully researched until about 40 years ago. But several studies have been conducted since at this time. The ideological belief in the herb, which is used as a cure for different diseases, is the reason for popularity of the plant. In fact, this plant has occupied special place for its wide range of medicinal value because of it might be found in the complex chemical composition of the seeds. The seed has over 100 different chemical constituents, including abundant source of all the essential fatty acids (Ramadan & Moresel, 2002a). This herb has been used to strengthen the immune system, cleanse the body, purify the blood, protect against irritants and support healthy longevity. Although their oil that most often used medicinally, and also used as a condiment in bread and other dishes (Merfort, I. et al, 1997), for the preparation of a traditional sweet dish, composed of black cumin paste, which is sweetened with honey or syrup, and in flavoring of foods, especially bakery products and cheese. *N. sativa* seed oil is considered as one among newer sources of edible oils, thanks to its important role in human nutrition and health (Salma, C.R et al, 2007). Black cumin has many nutritional and pharmaceutical uses. The seeds also can add to tea, coffee and breads, used in canning or extracted in wine or vinegar. In addition, most people take the oil in capsule form for seeking the benefits of black

cumin and also used for beauty as well as for treating skin conditions such as psoriasis and eczema. A mixture of oil with beeswax can be used for burns, skin infections, moisturizers, joint pain relived, or an anti-wrinkle agent (Al-Gaby, 1998). Now a day's black cumin oil can be extracted from the seed by different extraction methods such as using cold pressing, solvent extraction or supercritical CO_2 extraction methods or Steam Distillation.

Plants have been used as a source of medicine in Ethiopia from time immemorial to treat different aliments. Due to its long history, traditional medicine has in fact become an integral part of the culture. It is not unusual for people living in the countryside to treat some common aliments using plants available around them. Dawit Abebe reported that 80% of the Ethiopian populations depend on traditional medicine for their health care. More than 95% of traditional medical preparations are of plant origin (Dawit Abebe, 1986). A various uses of these seed and easily availability of the plant in our country we need to extract the oil and use for different purpose.

In commercial applications, solvent extraction method is commonly used for the extraction of fixed oil which produces higher yields, quicker and less expensive. The most common solvent is petroleum-derived hexane. This technique is used for most of the "newer" industrial oils such as soybean, black cumin and corn oils. In laboratory scale the apparatus that used for the extraction of fixed oil is soxhlet extractor. Although the oil of black cumin seed has a great uses for our health, the hexane solvent that used for extraction can cause air pollution during extraction and recovery, a trace of hexane in the oil that affect the quality of the oil and affect the health of the users.

The aim of this project is the extraction, optimization and characterization fixed oil of Ethiopian black cumin seed by using solvent extraction method, investigate the effects of the two solvents that used named as hexane and ethanol, seed solvent ratio and time, and in addition to that characterize the black cumin oil for different chemical and physical properties.

1.2. Statement of the problem

As we know Solvent Extraction method is considered as one of the most effective extraction techniques that would yield greater amount of oil than any other conventional methods and hexane has been commonly used as a solvent for the extraction of oil from black cumin seeds. However the use of hexane as a solvent causes a negative effect in human health and environment during extraction and recovery process. Therefore this thesis work aimed to use another alternative solvent named as ethanol where that hasn't any significant effect on the environment and human health for the extraction of black cumin oil.

There is a great effect of types of solvent on the extraction of fixed oil and still now hexane was the only solvent that used for extraction process. But because of the toxicity of hexane the researchers have to be concentrate to other alternative solvent and evaluate the effect of those solvent on the yield, physical and chemical properties of the fixed oil. So among those of alternatives solvent these studies select Ethanol and evaluate the effects compare to hexane solvent on the yield of the oil, physical and chemical properties of the fixed oil.

The reason to select Ethanol as an alternative solvent since its cost is low and it may be produced from a large variety of biological materials using simple technology. It can also be obtained by fermentation and therefore labeled as ''natural'' and also we can get from different liquor factories and sugar factories that produce ethanol. In addition, ethanol is recognized as non-toxic and has less handling risks than hexane. The use of this alcohol as an extraction solvent also avoids eventual toxicity problems of meals for animal feedstuff.

1.3. Objectives

1.3.1.General objective

The general objective of this research was to investigate and evaluate extraction of black cumin fixed oil from black cumin seed by using Hexane and ethanol solvents.

1.3.2.Specific objectives

- Investigation of the optimum operating parameters, extraction time, seed: solvent ratio
- Evaluate of the yield of the fixed oil
- Identify the components of the extracted oil by using GC-MS and FT-IR
- Characterize of the physical and chemical properties of the extracted oil
- Analyze the effect of solvent on the yield of oil

1.4. Scope of the study

This study is an experimental study to analyze the effect of hexane solvent for black cumin oil extraction in environment and health of human beings by replacing with ethanol solvent and doing the experiment using the two solvents to determine the efficiency of extraction starting from purchasing the available raw material. In addition, analyzed the effect of extraction time and seed: solvent ratio on the yield of the oil and also evaluate the optimum operating condition on extraction of black cumin oil yield and characterize the oil that will get from the experiment. By using GC-MS and FT-IR methods, determine the components and functional groups of that are found in the extracted oil and finally purify the oil and characterize the physical and chemical properties of both crude and refined fixed oils that extracted by the two solvents.

1.5. Significance of the study

This study is contributing a significant method of identification of high quality black cumin oil by analyzing the effect of solvents, time of extraction and seed solvent ratio using the process of solvent extraction.

In our country the numbers of commercial or large scale industries that are produce different types of oil that used for food, cosmetics and for pharmaceutical uses from different plants are not enough. Black cumin oil is the most important oil that is used for different purpose especially it is a medicine for different disease. This study will contribute for our country it is better to extract black cumin fixed oils from the seed by using solvent extraction method to get an optimum yield and minimize the cost of currency of both hexane chemical and the Black Cumin fixed oil that comes from foreign countries.

Environmental pollution control is the main essential element for the industries, companies, small enterprise's and every person. So replacing the Hexane solvent with environmental friendly solvent by Ethanol is an improvement of the process.

2. LITERATURE REVIEW

2.1. Overview of black cumin seeds

The scientific term of Black Cumin is Nigella Sativa comes from the Latin word, nigellus, meaning black and it is translated as "Seeds of Blessing". Black cumin has even been described as a "miracle herb". The seed is called black cumin or black seed in English. *Nigella sativa* is a member of the Ranunculacae family (Khan et al, 2011) and annual flowering plant. It grows to 20-30 cm tall, with finely divided, linear leaves. The flowers are delicate, and usually colored pale blue and white, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds. Nigella Seeds / Black Cumin Seeds are about the same size as sesame seeds, though they have a more triangular instead of oval shape. Nigella sativa seed is known variously as kalonji. In English it is called fennel flower, black caraway, nutmeg flower or Roman coriander. Nigella sativa has a pungent bitter taste and a faint smell of strawberries.

The growth area of Nigella sativa L. extends from the countries of the southern- and eastern-rim of the Mediterranean basin to Iran, Pakistan, South Europe, India, Syria, Turkey and Saudi Arabia. The plant may have originally been grown in in Ethiopia. And also it is native to Southern Europe, North Africa and Southwest Asia (Shewaye, 2011).

Figure 2-1: Black cumin seed

2.2. Constituent of the black cumin seed

Black seed herb contains over 100 components, the chemical composition is very rich and diverse and many of which still remain to be discovered. The Black Seed constituents that have been identified include: - Fixed oils (84% fatty acids:- Myristic Acid (0.5%), Palmitic Acid (13.7%), Palmitoleic Acid (0.1%), Stearic Acid (2.6%), Oleic Acid (23.7%), Linoleic Acid [Omega-6] (57.9%), Linolenic Acid [Omega-3] (0.2%), Arachidic Acid (1.3%)),

Volatile and essential oils, 15 Amino Acids (All essentials are present), proteins, Thiamin, Riboflavin, Pyridoxine, Niacin, Folacin, carbohydrates, Alkaloids, saponin, as well as minerals such as calcium, iron, Copper, Zinc, Phosphorus sodium, potassium and crude fiber. There are still many components in Black Seed that haven't been identified. But research is going on around the world.

Table 2-1: Constituent of black cumin seed

Constituent	Amount
volatile oil (yellowish)	(0.8 - 1.8%)
fixed oil	(27.6 - 41.6%)
Proteins	(13.5 – 20.5%)
Carbohydrate	(27 – 30.8%)
Fiber	(6 – 9%)
Ash	(3 – 8%)
Moisture	(4 – 6%)

Source: http://arabicherbalmedicine.com/?p=368

2.3. Uses of black cumin seed

Black Seed is an excellent herb with many benefits, especially when it comes to maintaining a strong and healthy immune system. Both the seeds and oil from the seeds are used as a nutritional supplement. Black seed, in its complete, natural form, acts on the principle of assisting the body's own natural healing process in overcoming illness or maintaining health. It works on the part or system of the body affected without disturbing its natural balance elsewhere.

Many studies show that, while black cumin seed is effective by itself, the effect combined with other nutritional and medicinal herbs is that not only does it help relieve the current condition at hand, but also helps the body build further resistance against future ailments or disease.

Because of its rich nutritional value, the use of black cumin seed as an energy source on ancient time. Still now, it believed that it is used to increase heat in the body, making metabolism more efficient. As a nutritional supplement in modern times, black cumin seed is used to treat respiratory conditions In addition, it is used to support stomach arthritis and circulatory complaints, allergies and hay fever, acne and intestinal health as well as kidney

and liver function. Several skin conditions can be treated with black cumin seed, and it is also used to enhance circulation.

Black Seed is a safe and excellent herb that can be used by anyone. It has no known side effects and has a long history of use for several thousand years. So daily uses of these seeds as:

- **As daily health supplement:** In cognizance of its substantial nutritional components, as well as its specific medicinal increased through regular use of black seed.
- **As an energy source:** The rich nutritional value contained in black seed points to it as a great source of energy. It has an ability to maintain and restore body heat.Black seed and other medication: Black seed may be used in conjunction with conventional or other forms of natural medicine. Black seed may be used as a therapeutic aid together with this and other forms of treatment to help counteract any side effects experienced from the use of antibiotics or other potent, chemically based medicines.
- **Pregnancy and lactation:** The black seed is not recommended during pregnancy; however during lactation It is an excellent form of added nutrition for both mother and the growing child while its immune system boosting properties serve as a natural, safe way to build resistance against illness. In addition, as studies have shown, black seed helps increase milk production during breastfeeding.
- **Black seed for the elderly person:** Which its rich nutritional, energy-giving value, in combination with immune system strengthening properties, black seed is an ideal health supplement for the elderly person.

2.4. Black cumin seed in Ethiopia

Black Cumin has a long history of uses for food flavors, perfumes and medicinal values. In Ethiopia, it is commonly used in the preparation of "Berbere" (in Amharic) in which it tends to reduce its hotness (Hedberg et al., 2003), for preparation of curries, bread, katikala,"Shamita" (Mogessie and Tetemke, 1995), traditional Ethiopian stews, "Wot" and preservation of butter. Black cumin is used principally to flavor food, either as whole grain, in powdered form or as an oleoresin extract. Within Ethiopia its main use is as a spice, which is typically ground and mixed with other spices. There is also some use in traditional medicine. The vast majority of Ethiopia’s black cumin exports go to Arabic countries, which, together with other predominantly Muslim countries, accounted in 2008 for some 98% of national exports. Sudan overtook Saudi Arabia as the main export destination in 2007 and by 2008 it accounted for almost one half of all official exports. It is uncertain how reliable this

market is and whether exports can be maintained at current levels. Value-adding to cumin in Ethiopia is low, with all exports being made in the form of whole grain (Orgut, 2007).

The annual production of black cumin seed in Ethiopia is around 18000 metric tons 2014/15 (Ethiopian Investment Agency, 2015). It is found in different parts of the country especially, in Kaffa zone, South West Ethiopia. There are 3 different seed varities named as Dirishaye, Eden, and Deribera Black cumin seed shows significant variations in days to flowering in the tested varieties at various locations. (Ermias Assefa, 2015; Alemu Belay, 2016)

2.5. Oil Extraction methods

The term “extraction” is derived from the Latin word “extrahere”; “ex” specifies the direction, i.e. out of, and “trahere” describes the action, namely drawing or removing. Extraction is defined as the process of removing a substance or several substances from another substance. The process is extremely important in a wide range of technical applications, for instance biotechnology, the pharmaceutical and food industries as well as environmental protection. Extraction is a separating process which has the advantage of low energy consumption. In numerous areas of application, extraction is the more efficient, more selective and less expensive alternative compared with competing separating methods such as distillation, evaporation and membrane technology. Extraction is the process of separation of medicinally active substances of plant or animal from a mixture by a mechanical or chemical action such as by distillation or pressure. This separation is done with the help of dissolving one or more of the substances in a solvent in which it is easily soluble and is separated on the basis of their physical or chemical properties. Extraction is the withdrawing of a active agent or a waste substance from a solid or liquid mixture with a liquid solvent. The solvent is not or only partial miscible with the solid or the liquid. By intensive contact the active agent transfers from the solid or liquid mixture (raffinate) into the solvent (extract). After mixing the two phases are separated which happens either by gravity or centrifugal forces.

2.6. Overview of Extraction methods

There are basically four types of oil (Essential oil and Fixed oil) extraction methods as represented in Figure below. (Source: Pelin Sari, 2006)

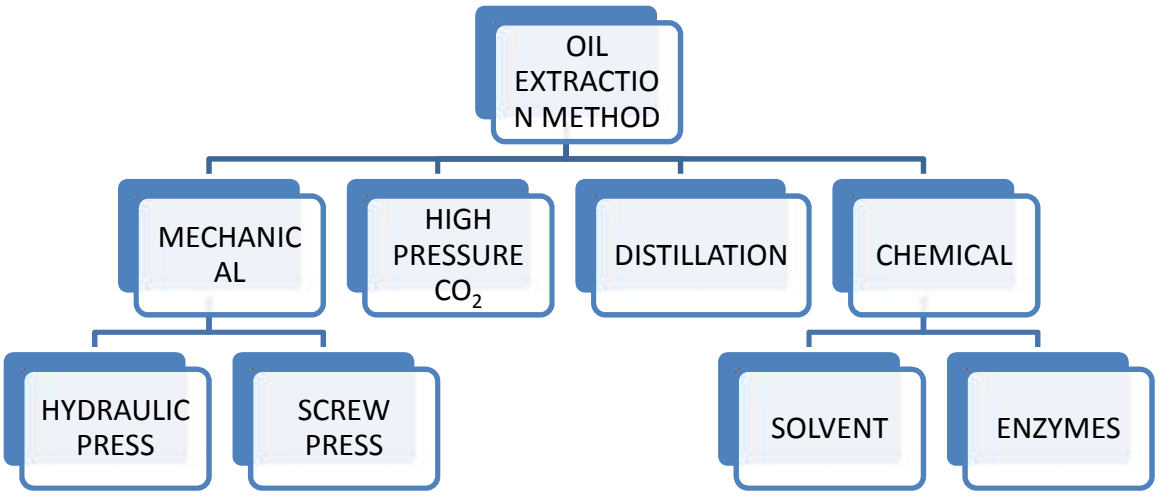


Figure 2-2: Basic oil extraction methods

2.6.1.Chemical extraction

Enzyme extraction

The oil extraction process by using enzymes is implemented by big vegetable oil companies because the process produces many high value products. The seeds are cooked and put into water. Enzymes are then added which digest the solid material. The basic difference of this type of extraction method from the solvent type is that the residual enzymes in the oil are separated by the use of a liquid-liquid centrifuge.

Solvent Extraction

Solvent extraction is a method of extraction that the use of solvents, such as petroleum ether, methanol, ethanol, or hexane, to extract the odoriferous lipophilic material from the raw material. The solvent will also pull out the chlorophyll and other plant tissue, resulting in a highly colored or thick/viscous extract. In the Solvent-Extraction method of fixed oil from the seed, grinding process of the seeds is necessary, because the contact area of the seed with the solvent should be maximized in order to increase the oil yield. Then the seed is loaded with perforated trays and repeatedly washed with the solvent. All the extractable material from the seed is dissolved in the solvent. This includes highly volatile aroma molecules as well as non-

aroma waxes and pigments. Then separate the extractable material from the solvent using rotary evaporator by heating the mixture up to the boiling point of the solvent.

2.6.2.Effect of solvent on Extraction

Various methods have been used from the previous researchers to extract the oil from black cumin seeds such as cold press method (Lalas S, Tsaknis J, 2002), supercritical carbon dioxide extraction (Palafox J O. et al, 2012), aqueous enzymatic method (Abdulkarim S, et al, 2005) and solvent extraction method (Adejumo B A, et al, 2013). In this investigation, oil extraction using the method of solvent extraction was carried out using hexane and ethanol as the solvent.As we mentioned before There are many factors influencing the oil extraction yield such as the method of extraction, temperature of extraction, seed particle size, ratio of solvent to the seed powder, pre-treatment conditions and residence time between the solvent and the seed (Mani S, et al, 2007). However, this research are only focusing on the organic solvent, ratio of seed powder to the solvent and contact time of the solvent with the black cumin seeds to increase the oil extraction yield. Therefore, the main objective of this research is to evaluate the suitable set of parameters in obtaining the highest oil recovery within the studied range from black cumin seeds. However, there are limited data on the processing factors needed for the ideal extraction of *black cumin* oil especially for the type of solvent used.

In history of solvent extraction most of the oil can be extracted from different seeds by traditional method and Water is a common traditional solvent used during extractions. First the seeds from mature pods are roasted, crushed and placed in boiling water for 5 minutes. After straining and left for overnight, the oil floats to the surface (Price M L, 2007). However carrying out this traditional method is not efficient as it is highly energy intensive and time consuming and only can produce low percentage of oil yield. Therefore, the researchers come up with different solvents to maximize the oil recovery from different seeds.

Ideally, one selectively extracts the target compound by using a solvent whose polarity is close to that of the target compound. Volatile solvents such as hexane, benzene, ether, ethyl acetate, and dichloromethane are usually used for the extraction of semi-volatile compounds from water. Hexane is suitable for extraction of non-polar compounds such as aliphatic hydrocarbons, benzene is suitable for aromatic compounds, and ether and ethyl acetate are suitable for relatively polar compounds containing oxygen. In addition to that polar solvents such as methanol and ethanol, or mixtures of polar and non-polar solvents whose boiling points are close to those of ethanol / benzene, or acetone / hexane are used as a solvent.

Presently n-hexane is the preferred solvent throughout the world due to its extraction efficiency and ease of availability. But this is not the best method for extraction of oil as the hexane can leave a small amount of residue behind which could cause allergies and effect the immune system and during extraction and recovery of Hexane can be emitted and has been identified as an air pollutant since it can react with other pollutants to produce ozone and photochemical oxidants (Wan *et al.,* 1995a; Hanmoungjai *et al.,* 2000). Hexane has been categorized as a hazardous air pollutant (HAP) by the US Environmental Protection Agency and is included in the list of toxic chemicals (NIOSH. 2007). The maximum permissible limit for n-hexane in oil and the meal are 5ppm and 10ppm, respectively (PFA act 1954). It is very tedious and energy consuming to reduce hexane concentration in the meal up to or below maximum permissible limit. These problems have attracted researchers to find a suitable alternative solvent.

A number of solvents and their mixtures like heptane, acetone, ethanol-water azeotrope, methyl-pentane, isohexane, petroleum-ether, trichloroethane, chlorinated hydrocarbons, alcohols etc. for oil extraction from oilseeds have been reported in literature (Conkerton *et al*., 1995; Wan *et al*., 1995(a) and (b); Gandhi *et al*., 2003; Maria *et al*., 2008; Kuk. *et al*., 2005; Kuk, 1998; Sepidar *et al*., 2009; Rittner, 1992; Hron *et al.,* 1994; Sineiro *et al.,* 1998). Among hydrocarbon solvents, heptane and iso-hexane were recommended as potential substitutes for hexane to extract oil from cottonseed (Wan *et al.,* 1995a; Wan *et al.,* 1995b; Conkerton *etal.,* 1995). With respect to the use of alcohols, isopropanol and ethanol are the most promising solvents for the oil extraction from cottonseed (Hron *et al.,* 1994), sunflower seed (Sineiro *et al.,* 1998) and soybean (Baker and Sullivan, 1983; Rittner, 1992). Out of these, use of alcohols is gaining attention due to their higher threshold limit in the environment. Junfung (2010) has reported simultaneous extraction of oil and very good results have been reported by him for extraction of oil. Ethanol is a worthy candidate to investigate as an alternative solvent since its cost is low and it may be produced from a large variety of biological materials using simple technology. In addition, this alcohol is inflammable (flash point= 8.9 ^{0}C; ignition temperature= 425 ^{0}C), is recognized as non-toxic and has less handling risks than hexane (flash point = -23 ^{0}C; ignition temperature= 225 ^{0}C) (Rittner, 1992). In the alcohol series, ethanol is the safest solvent as it is obtained from biological sources by the fermentation process and is placed in the category of GRAS (generally recognized as safe). Very few studies are reported for the use of aqueous ethanol as solvent. Hron *et al*., (Hron *et al*., 1994) have reported a two-step process using 95% ethanol for extraction of oil from cottonseed. The extraction process consists of extraction at

lower and higher temperatures; the lower temperature facilitates the reduction of gossypol concentration in the meal while higher temperature facilitates oil extraction. No detailed data are available in the literature regarding oil extraction from black cumin seed with absolute ethanol. Therefore, the study has been conducted to compare the efficiency of oil extraction from black cumin seed by n-hexane and ethanol at temperature of their respective boiling point to explore the possibility of using ethanol as a solvent to obtain oil. This paper discusses the effect of parameters viz. solid ratio - solvent, extraction time on the oil extraction efficiency.

2.7. Fixed oils

Fixed oils are extracted from a wide variety of plants; the part of the plant that is extracted for oil depends on the type of plant. Some plants store oil in their leaves or flowers whereas others store oil in their seeds. Fixed oils are also commonly referred to as vegetable or base oils. They are the oily – nonvolatile part of the plant, typically obtained from the seed or nut. They are called ‘fixed’ as they have large molecules that do not evaporate like the essential oils. (http://naturalhealthezine.com)

Carrier oils are mixtures of unsaturated and saturated fatty acids with traces of vitamins and minerals along with a variety of other trace plant constituents. The type of nutrient you acquire depends on the type of fixed oil used. Most fixed oils lack their own scent or aroma. And if they do contain such aromas, it is usually very subtle. This quality is important since fixed oils are often combined with essential oils during aromatherapy application to avoid competing with the latter's aroma. As you know essential oils should not be applied directly to the skin (the term ‘neat’ is used when essential oils are not diluted and used on the skin). They should be diluted into a carrier. Carriers can be water based or oil based. The base you choose will depend on the final use of the intended product. Fixed oils are extracted usually by a process called Cold Press or Expeller pressing. Some are extracted using solvents, heat and/or steam.

Despite the application on other industries, it is generally limited to the practice of aromatherapy. This is due to the properties contain in fixed oils that enable them to provide natural skin care treatment. However, it is also largely used in other industries such as food, toiletries, or when making aromatherapy oil blends. Different fixed oils work better for some skin types including variations due to the age of skin such as infants, teens and the elderly.

Also there are carrier oils that will work better on oily, dry or combination skin. Of course massage and skin ailments will also be a factor as to the choice of fixed oil you might use. These oils are the “bases” used to make massage oils, bath oils and can be used in bath salts and scrubs. Several of them can be added to shampoos and conditioners to make your hair feel and look better. Many of them are found in lotions and creams. All of them are used in the cosmetic industry.

Carrier or fixed oils are often more fragile than their essential oil counterparts. Hence, we need to observe proper storage options if we intend to store them over a given period of time. Here are some important guidelines to store and preserve our fixed oils for a longer use:

- Use dark colored glass bottles for storing carrier or fixed oils, such as with essential oils. Make sure to use tight fitting tops to keep the oils enclosed and avoid other environmental substances from entering the bottle and spoiling the oil.
- We can also opt to use plastic bottles when storing fixed oils, something of which is not allowed when storing essential oils. However, this is probably especially if you intend to use fixed oils shortly upon purchase.
- To prolong the shelf life of carrier or fixed oils, we can store it inside the refrigerator. One such example is borage seed oil. Avoid storing avocado oil inside your refrigerator, however. If we store the fixed oil inside a refrigerator, it can solidify. So, take out the oil from the refrigerator a few hours before you intend to use it to allow it to return to room temperature and go back to its liquid form.

2.7.1.Physical properties of fixed oil

The specific gravities of oils vary between the limits of 0.910 and 0.975. The lowest specific gravity is owned by the oils belonging to the rape oil group - from 0.913 to 0.916. The specific gravities of most non-drying oils lie between 0.916 and 0.920, and of most semi-drying oil between 0.920 and 0.925, whereas the drying oils have specific gravities of about 0.930. The fixed oils are practically insoluble in water. They are completely soluble in ether, carbon bi-sulphide, chloroform, carbon tetrachloride, petroleum ether, and benzene. Oils have no distinct melting or solidifying point. The freezing-points of those oils which are fluid at the ordinary temperature range from a few degrees above zero down to - 28° C. (linseed oil). At low temperatures solid portions - usually termed "stearine" - separate out from many oils; in the case of cotton-seed oil the separation takes place at 12° C. These solid portions can be

filtered off, and thus are obtained the commercial "demargarinated oils" or "winter oils." Oils can be heated to a temperature of 200°c to 250° C without undergoing any material change, provided prolonged contact with air is avoided. On being heated above 250°c up to 300°c some oils, like linseed oil, safflower oil, tung oil (Chinese or Japanese wood oil) and even castor oil, undergo a change which is most likely due to polymerization. In the case of castor oil solid products are formed. Above 300°C, all oils are decomposed; this is evidenced by the evolution of acrolein, which possesses the well-known pungent odour of burning fat.

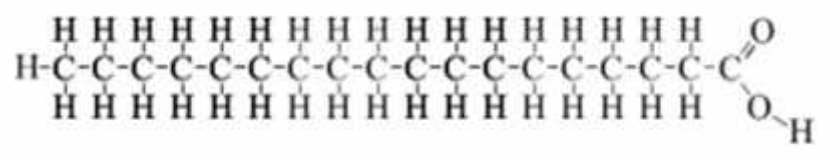
On exposure to the atmosphere, oils gradually undergo certain changes. The *drying* oils absorb oxygen somewhat rapidly and dry to a film or skin, especially if exposed in a thin layer. Extensive use of this property is made in the paint and varnish trades.

The *non-drying* oils, the type of which is olive oil, do not become oxidized readily on exposure to the air, although gradually a change takes place, the oils thickening slightly and acquiring that peculiar disagreeable smell and acrid taste, which are defined by the term "rancid." The changes conditioning rancidity, although not yet fully understood in all details, must be ascribed in the first instance to slow hydrolysis ("saponification") of the oils by the moisture of the air, especially if favored by insolation, when water is taken up by the oils, and free fatty acids are formed. The fatty acids so set free are then more readily attacked by the oxygen of the air, and oxygenated products are formed, which impart to the oils the rancid smell and taste. The products of oxidation are not yet fully known; most likely they consist of lower fatty acids, such as formic and acetic acids, and perhaps also of aldehydes and ketones. If the oils are well protected from air and light, they can be kept indefinitely.

2.7.2.Chemistry & Structure of Fixed Oils

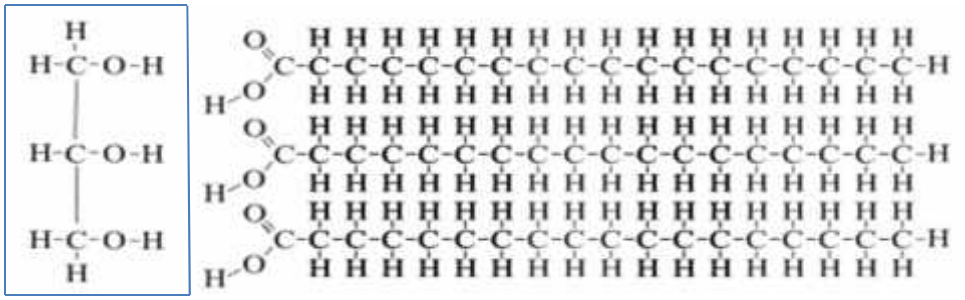
Fixed oils from seeds, nuts & vegetables are typically composed of triglyceride molecules. A triglyceride is typically composed of a 3-carbon alcohol (glycerol) plus three 18-carbon (or 16-carbon) fatty acids. The 18-carbon fatty acids are Linoleic acid, Stearic acid & Oleic acid.

- Stearic Acid Saturated: All Single Bonds Between Atoms Of Carbon



- Glycerol + Three Fatty Acids = A Fat Molecule (Triglyceride)

In the reaction below, 3 molecules of fatty acid (stearic acid) combine with 1 molecule of glycerol, with the removal of 3 molecules of water. Note that both glycerol and carboxylic acids have -OH bonds, which together contribute 2 hydrogen atoms and 1 oxygen atom to form the water. Three ester linkages are formed as a result.



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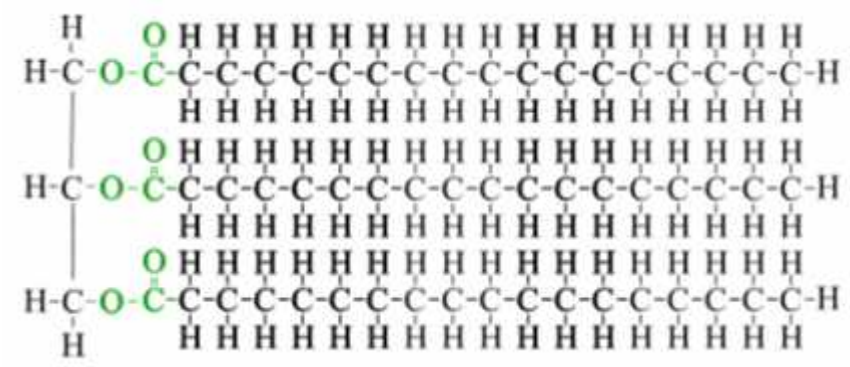
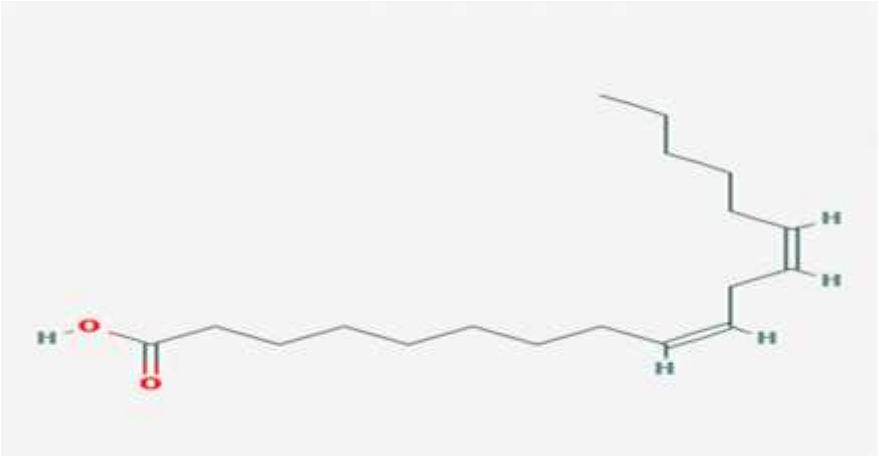
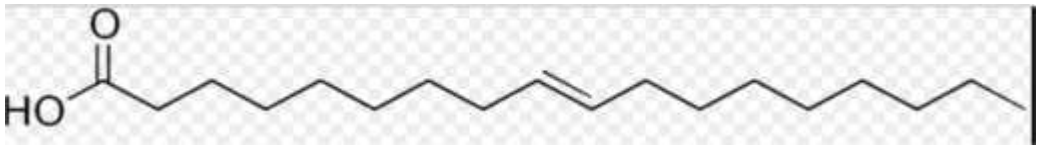


- Linoleic Acid Polyunsaturated: 2 Double Bonds In The Molecule



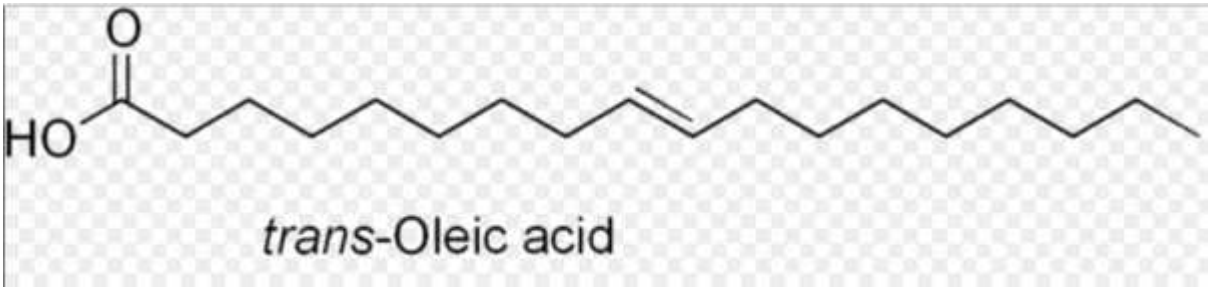
- Oleic Acid Monounsaturated: 1 Double Bond between Carbons 9 & 10



The fatty acids may be saturated (with all single bonds), mono-unsaturated (with one double bond) or polyunsaturated (with 2 or more double bonds). Plant fatty acids are usually unsaturated and liquid at room temperature, with one or more double bonds between the carbon atoms (mono-unsaturated and polyunsaturated). A notable exception is the palm fatty acid – palmitin – which is saturated and contains 16 rather than 18 carbon atoms. Since the plant fatty acids are unsaturated, the plant oils it is liquid at room temperature.

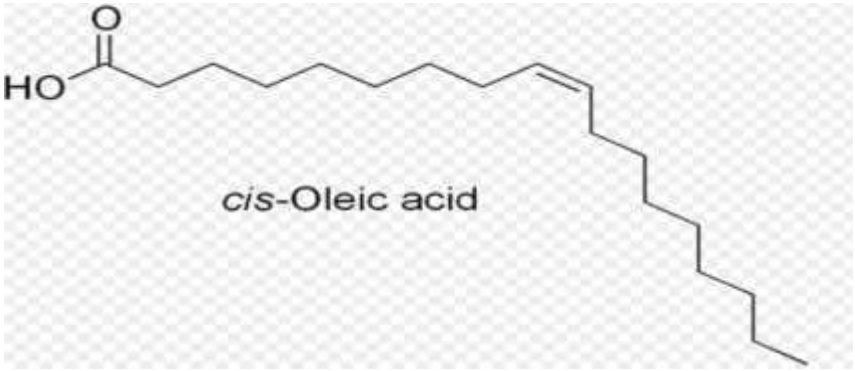
Cis & Trans Fatty Acids

Fatty acid isomers containing double bonds may have the cis or trans configuration. In cis fatty acids, all the hydrogen atoms adjacent to the double bonds are on the same side of the longitudinal carbon axis. In trans - fatty acids, the hydrogen atoms adjacent to the double bonds occur on alternate sides of the main axis. The trans configuration is chemically more stable. It is typically produced during partial hydrogenation of polyunsaturated vegetable oils. Trans fatty acids tend to raise the level of low density lipoproteins (LDLs = bad) and lower the level of high density lipoproteins (HDLs = good). These changes in blood lipids (cholesterol levels) may increase the risk of heart disease (atherosclerosis) in some people. Dieticians generally recommend the use of mono-unsaturated, unhydrogenated oils and the avoidance of trans-fatty acids found in French fries, cookies and crackers.



Unsaturated fatty acids found in plant oils and seeds are typically omega-6 fatty acids, in which the first double bond is located on the sixth carbon atom, counting backwards from right to left. Omega-3 fatty acids; the first double bond in on carbon #3 - are prevalent in fish

oils and flax seeds. (Plant Oils - Vegetable Oils, Oilseeds, Eessential Oils and Plant Based Oil Resources and Links - CastorOil.in.html)



2.7.3.Rancidity of Fixed Oils

The proper storage options for carrier or fixed oils as described in the above because of most of these oils turn rancid quickly. Rancidity of Fixed Oils occurs due to the presence of various substances such as natural fatty acids and tocopherols, effects of extraction methods, and the overall characteristic of the oil that is different from essential oil. Which also accounts to the period it takes for an oil to become rancid (https://hubpages.com).

If the fixed oil emits an aroma that is stronger than usual, since it may be already go rancid. At this point, you would no longer want to use the oil since it can potentially be harmful. If possible, compare the aroma of a fixed oil you suspect to be rancid against another oil of the same botanical source that is relatively new. For added assurance, go for fixed oils that are unadulterated and is extracted only from natural sources.

2.7.4.Refining of the Fixed oil

Fixed oils as received from the extraction plant contain several non-triglyceride components which must be removed. Refining consists of several processes which accomplish this aim. A refining process is carried out following extraction of fixed edible oils by means of screw presses and/or solvent extraction. In refining, physical and chemical process are combined to remove undesirable natural as well as environmental – related components from the crude oil. These components comprises for example phosphatides, free fatty acids, pigments (such as chlorophyll), odors and flavors (including aliphatic aldehyde and ketone), waxes as well as heavy metals, pesticides and so on. Figure 2-3 shows that the process of oil refining that starting from degumming up to final purifying stages.

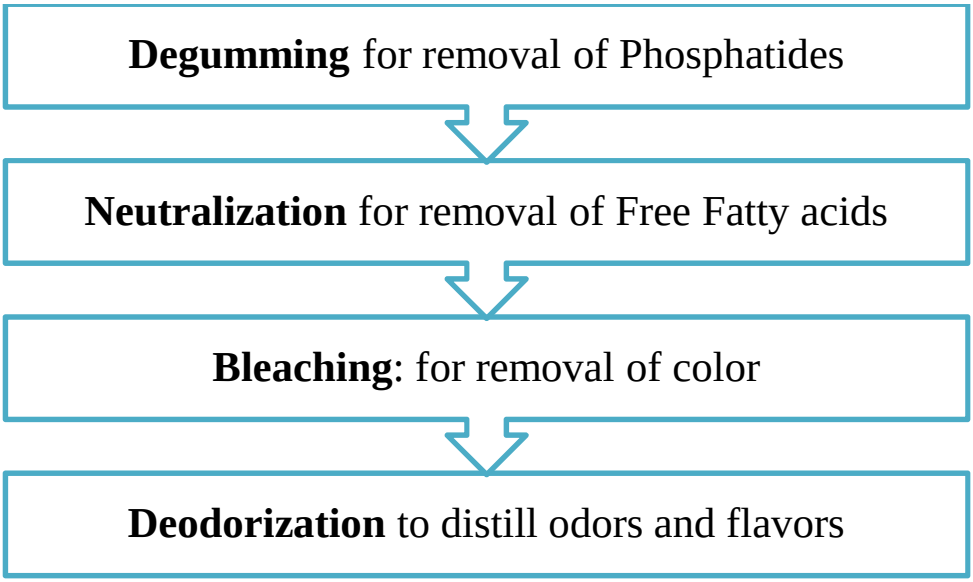


Figure 2-3: Oil refining process

Degumming

Crude oil obtained by screw pressing and solvent extraction of oilseeds will throw a deposit of so-called gums on storage. The chemical nature of these gums has been difficult to determine. They contain nitrogen and sugar and can start fermenting so they were at one stage thought to consist of glycolipids and proteins. The gum consists mainly of phosphatides but also contain entrained oil and meal particles. Degumming is the first stage in the refining process. It is used to separate the gums, phospholipids, and proteins that are insoluble in oil when hydrated. There are two main types of gums that need to remove from the extracted oil. The first one is Hydratable phosphatides, that are easy to remove by using only water and the other is Non Hydratable Phosphatides (NHP) that are hard to remove from the oil. Some of the NHP removed with hydratable in water degumming and the other is required the use of acid (Phosphoric acid) to convert to hydratable for complete removal.

Water degumming

A large part of the phosphatides (gums) can be hydrated quickly and easily. If the extracted oil contains a considerable quantity of gums the oil is subjected to the water degumming process immediately following extraction. Hydrating the gums and removing the hydrated gums from the oil before storing the oil can prevent the formation of a gum deposit. This treatment is called water degumming. Water degumming treatment forms a certain phospholipids, such as lecithin, find widespread industry application. Since the water degumming process involves more water than when crude oil is allowed to absorb moisture

from the atmosphere, the gums resulting from the water degumming process also remove hydrophilic substances such as sugars from the oil. In this process, water is added to the oil. After a certain reaction period the hydrated phosphatides can be separated either by decantation (settling) or continuously by means of centrifuges.

Chemical refining and neutralizing process

In this process stage, the phosphatide portion of oil is either removed or conditioned during this stage by addition of some additives/agents under specific condition. The most common additives for this purpose are phosphoric acid and citric acid.

A specified quantity of any one or mix of such additives/agents is mixed in the oil charge for a specific period and process parameters. This causes a separation of phosphatides from oil and they are removed after certain settling time. Sometimes these separated gums are not directly removed but with soap stock formed during neutralization.

The oil mass is then neutralized with alkali (NaOH) for removal for free fatty acid in the form of soap stock. This soap stock is removed from oil mass by gravity separation method. For removal of alkali traces, oil is washed with hot water. The chemical reaction involved in this operation is as follows:

$$\mathbf{R\text{-}COOH + NaOH \longrightarrow RCOONa + H_2O}$$

Bleaching Process

The bleaching of oils is a part of the refining process of crude oils, which removes coloring that adversely impact the appearance and performance of this triglyceride (triacylglycerol)-based materials. Typically, oils are extracted together with impurities in various quantities. Many of these impurities have to be removed from the oil to achieve the high quality oil standards necessary for edible applications. Preceded generally by degumming and refining (neutralization) processes, bleaching is required to remove specific detrimental contaminants that are not effectively removed by these processes before the oil progresses through deodorization.

Originally described as a process of mixing oil and clay adsorbent to remove color, the bleaching operation effectively removes some of the color, reduces the contents of chlorophyll, residual soap and gums, trace metals, oxidation products, and indirectly impacts on deodorized oil color by using bleaching clay (bleaching earth).

Deodorization

The oil after bleaching is practically pure, but contains minute quantities of original odoriferous matter and also the chemicals used during neutralization process. This bleached oil is then sent to a cylindrical vessel called `Deodorizer'. The Deodorizer is kept under very high vacuum and the bleached oil is then heated at high temperature 200^0C with high-pressure steam and open steam is passed through the oil. The volatile materials are evaporated off with some carrier (Commonly direct steam). This oil is then cooled and clarified through a Filter Press to get sparkling oil. The purpose of deodorization is to make oil blend and tasteless. In this process, the peroxide value of oil is brought down as minimum as possible.

2.8. Black cumin seed oil

The acceptance of the people's consumption on herbal based products especially both fixed and essential oil is getting high because of the numerous beneficial therapeutic impacts. They could give to our body and indirectly helps us sustaining a healthy condition. In order to combat a lot of chronic diseases, it is common to use artificial and synthetic drugs from the market, but these medications usually have negative effects to our body. So, people start to realize the differences between traditional and modern medication technique. Therefore, if consumed accordingly based on scientific findings and research, products from herbs, are the right choice in treating certain kinds of diseases without introducing side effects to our body. On their own standards both the herb and the oil of the black cumin seed (Nigella Sativa) are effective but the oil of black cumin is more concentrate than the herbs. Black cumin (Nigella sativa L.) contains several bioactive molecules that include thymoquinone, thymol, tocopherols, tocopherol, trans-retinols, and selenium. These functional ingredients are predominantly present in its fixed and essential oil (sultan MT, et al, 2009). So extraction of fixed oil from the seed of black cumin seed is better for use for different purpose. The primary measure of the oils potency and nutritional value is its purity. This in turn is determined by the quality of the raw Black Cumin Seeds and especially the actual extraction process of the pure oil. Different researcher's would be recommended that, A teaspoon of Black Seed Oil or the seeds be taken daily as a dietary supplement. The oil does not taste nice, but it can be mixed into a glass of orange juice or into yoghurt to camouflage its taste.Like most herbs, the composition of black cumin varies with the geographic distribution, time of harvest and agronomic practices. Black Seed Oil contains several ingredients (in significant amounts) with potential value. The original "Black Seed" brand of

Black Cumin (Nigella sativa) seed oil is a valuable source of protein, carbohydrates, essential fatty acids, vitamins A, Bi, B2, C and niacin as well as minerals such as calcium, potassium, iron, magnesium, selenium and zinc. The following chart reflects the composition of Black Seed Oil in terms of its active, nutrient components, and any other significant ingredients.

2.8.1.Phsyco-chemical properties of black cumin oil

The Physico- chemical properties of black cumin seeds fixed oil extracted by different solvents have been explained by different researcher s. The solvent extraction of N. sativa gives a strong aromatic odor and has been characterized by more yellow dark color than other vegetable oils and they can protect against UV light. the presence of greater amounts of thymoquinone is the reason of the yellow dark coloration, which could be responsible for the color in addition to what was suggested by S. Cheikh-Rouhou et al(Cheikh-Rouhou S. et al, 2007); ‘‘the coloration is due to the presence of more yellow pigments of carotenoids and linoleic acid’’. Indeed, at -4^0C during 72hr, the thymoquinone isolated from the volatile oils showed that this compound is black, and when it is dissolved in hexane we note the dark yellow coloration of the solution formed.

The investigation of the yield in most Nigella seeds oil is higher than 30% (Akram Khan, 1999 and Matthäus and Özcan, 2011) and possibly up to 40 %(Cheikh-Rouhou et al., 2007). However, the yield is reduced up to 13–23 if the phenological events as water-stress or saline conditions, and cool temperature(Al-Kayssi et al., 2011, Bourgou et al., 2010 and Atta, 2003). Comparing to the cold press-extracted Nigella seed oil, the solvent-extracted oil has already been observed higher yield and explained the high solvent efficiency to extract oleoresins, compared to cold-pressing (Atta, 2003).

Table 2-2: Yield of black cumin oil extraction process

Country	Yield (%)	Reference	Country	Yield (%)	References
Morocco	37	Said Gharby et al.(2013)	Egypt	34.8	Ali and Blunden (2003)
Italy[a]	13–23	D'Antuono et al. (2002)	Iran	40	Cheikh-Rouhou et al. (2007)
Tunisia	28	Cheikh-Rouhou et al. (2007)	Turkey	32	Nergiz and Ötles (1993)
	31.7	Hamrouni-Sellami et al. (2003)		30–36	Matthäus and Özcan (2011)
Pakistan	31.2	Sultan et al. (2009)	Bangladesh	32	Ali et al. (2012)
Turkey	29.4–29.7	Üstun et al. (1990)	Saudi-Arabia	38.2	Al-Jassir (1992)
Yemen	36.8–38.4	Al-Naqeep et al. (2009)			

Source: (Said Ghabry, et al, 2013)

Specific gravity values of oils are less than 1 for most of the oils except few containing oxygenated aromatic compounds (Bamgboye AI, Adejumo OI, 2010). Different researchers shown that specific gravity for the black cumin seed fixed oil which extracts by different solvents were 0.9194 - 0.9473 (gm/ml) at 25^0C(M. Abbas Ali et al., 2008). Another study by Al-Bahtiti (Al-Bahtiti NH, 2015) found that the specific gravities estimated with N. sativa 0.9071 at 25^0c.

The range of refractive index (RI) for the fixed oil that was explained by (Essam F. Al-Jumaily and Anwar Jalil Shihab Al-Jumaily, 2015) 1.341-1.466 at 20^0C and that depends upon extracted methods and the temperature. Similarly that refractive index of the oils were found to be 1.4683 for Jordanian N. sativa at 30^0C founded by Al-Bahtiti (Al-Bahtiti NH, 2015), Generally Refractive index of N. sativa is consistent with the reported values 1.4600-1.4700 at 40^0C for the same seed oil (Salma, C.R., et al. 2007)). The viscosity (cSt) for the fixed oil for Nigella seeds which was already explained by (Essam F. Al-Jumaily and Anwar Jalil Shihab Al-Jumaily, 2015) extracts by different solvents, around 40.65 - 54.38. Iodine values estimated for N. sativus 115 (M. Abbas Ali et al., 2008)and 119 reported by Salma et al (Salma et al., 2007). And also describes (Said Gharby et al., 2013) Cold press- and solvent-

extracted Nigella seed oil presented a similar iodine value (126–128 g of I_2/100 g). The investigated saponification value for N. sativus is 211-218 mentioned by Salma et al. (Salma, C.R., et al., 2007), and also around 203 mentioned by Atta (Atta, 2003) for the seed oil.

2.8.2.Chemical composition of black cumin oil

Table 2-3: Chemical Compositions in Black Seed Oil

Essential Oil Composition (1.4%)	**Black Seed Oil**
Carvone	21.1%
Alfa-Pinene	7.4%
Sabinene	5.5%
Beta-Pinene	7.7%
P-cymene	46.8%
Others	11.5%
Fatty Acids	**Black Seed Oil.**
Myristic Acid (C14:0)	0.5%
Palmitic Acid (C16:0)	13.7%
Palmitoleic Acid (C16:1)	0.1%
Stearic Acid (C18:0)	2.6%
Oleic Acid (C18:1)	23.7%
Linoleic Acid (C18:2)(Omega-6)	57.9%
Linolenic Acid (18:3n-3) (Omega-3)	0.2%
Arachidic Acid (C20:0)	1.3%
Saturated & Unsaturated Fatty Acids	**Black Seed Oil**
Saturated Acid	18.1%
Monounsaturated Acids	23.8%
Polyunsaturated Acids	58.1%
Nutritional Value	**Black Seed Oil**
Protein	208 ug/g
Thiamin	15ug/g
Riboflavin	1 ug/g

Pyridoxine	5ug/g
Niacin	57 ug/g
Folacin	610 IU/g
Calcium	1.859 mg/g
Iron	105 ug/g
Copper	18 ug/g
Zinc	60 ug/g
Phosphorus	5.265 mg/g

Nutritional Composition	**Black Cumin Seed** /
protein	21%
carbohydrates	35%
fats	35-38%

Source: http://blackseedinc.stores.yahoo.net/chemanofblac.html

As we can see the table Black Cumin seed is rich in nutritional values. It has Monosaccharides components, rich in fatty acids, particularly the unsaturated and essential fatty acids (EFAs) especially alpha-Linolenic acid (omega-3) and Linoleic acid (omega-6), are substances that cannot be manufactured in the body. Among the Fifteen amino acids that make up the protein content of the Seed, eight of the nine essential amino acids cannot be synthesized within our body in sufficient quantities and are thus required from our diet. It is also contains Arginine which is essential for infant growth and carotene, which is converted by the liver into vitamin A. so in order to sustain our health we must use these seed in our everyday diet.

2.8.3.Fatty Acid Composition

Gas chromatography used to identify the constituents is identifying by matching their result and by comparison of their retention indices with literature values (Adams RP., 1995). Retention indices can be determined by using retention times of standard fatty acids that have been injected to the same instrument and under the same chromatographic conditions. There are a number of fatty acids that is found in Nigella sativa L. seeds oils. Farid Benkaci-Ali *et.al,* describes a total of 11 different methyl ester acids were identified by GC and GC-MS in the Nigella sativa seed oils (Farid Benkaci-Ali *et al., 2012).* The major saturated fatty acids were palmitic (C16:0), stearic (C18:0), and heneicosanoic acid (C21:0); the main unsaturated

fatty acids, linoleic (C18:2n6) and oleic (C18:1n9) acids represents and which account for 78.94% at 87.42% in Isopropyl Alcohol extraction oils and 74.09% at 81.95% in Hexane extraction oils. And they also checked the ratio of unsaturated to saturated fatty acids (U/S) that used to determine potential benefits on cognitive function and behavior, and inflammatory conditions such as arthritis, Crohns disease, and asthma if the value is higher(A. P. Simopoulos, J. Am. Coll. Nutr, 2002). The U/S value was higher in IA (IA: 5.41 to 8.66) compared to H (5.04 to 5.8). These ratios were higher than those reported by Ramadan et al (M. F. Ramadan *et al., 2003)* and Cheikh-Rouhou et al (Cheikh-Rouhou et al., 2007), which vary from 3.41 to 3.8. Bahman Nickavar *et al (*Nickavar B.*et al.,2003)* identified eight fatty acids in the extract, was consisted of four saturated fatty acids (17.0 %) and four unsaturated fatty acids (82.5 %). Linoleic acid (55.6 %), oleic acid (23.4 %), and palmitic acid (12.5 %) were the major components that are similar to literature values of Houghton *et al.* (Houghton et al., 1995).

(Essam F. Al-Jumaily and Anwar Jalil Shihab Al-Jumaily, 2015) describes the seed contain many fatty acids such as stearic acid (28.59%), linoleic acid (0.022%) and oleic acid concentration found 2.748% when extracted by n-hexane solvent. Nickavara et al. (Nickavara et al., 2003) found that Nigella sativa seeds form Iran contained form main fatty acids linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%).

The Free Fatty Acid (FFA) content in N. sativa seed oil (12%) that described by M. Abbas Ali et ai.(Abbas Ali M. et al., 2011), that was similar to the value 11% cited in the literature (Atta, 2003). And also they determine the unsaponifiable matter of the seed oil contained around 1.2%. The peroxide value of N. sativa is within the range of 10.7-13.5 revealed by Atta (Atta, 2003), but much higher than 4.3-5.6 reported by Salma et al. (Salma et al., 2007) for the same oil.

2.8.4.Uses of black cumin oil

Plants are natural factories for the production of chemical compounds, many of which are used to promote and fight diseases and some of them are marketed as food or herbal medicines (Dubick, 1986). Herbal medicines have long been viewed as a source of curative remedy based on religious and cultural traditions (Huxtable, 1992; Ghazanfer, 1994). Black cumin seeds are one of the earliest cultivated plants in human history that have been used as herbal medicine by various cultures and civilizations to treat and prevent the number of diseases to ensure good health.to keep people super healthy – for over 3,300 years.

Ancient Egyptians such as Cleopatra used it as its health beauty giving quality. Queen Nefertiti used black seed oil to bring luster to her hair and nails. Hippocrates used it to assist with digestive and metabolic disorders. King Tutankhamen kept a bottle of black seed oil in his tomb, presumably to protect him in the afterlife. Physicians in Pharaohs‟ times used black seed as a remedy for colds, headaches, respiratory and digestive disorders, toothaches, infections, inflammatory disorders and allergies (GreenMedInfo January 3, 2013).

The health enhancing potential of black cumin has been attributed to the active ingredients that are mainly concentrated in fixed and essential oil (Ramadan, 2007). The oil of *N. sativa* is so beneficial due to its content of over a hundred components such as aromatic oils, trace elements, vitamins and enzymes. It contains 58% of essential fatty acids including omega 6 and omega 3. These are necessary for the forming of Prostaglandin E1 which balances and strengthens the immune system giving it the power to prevent infections and allergies and control chronic illnesses. Healthy cells are protected from viruses thus inhibiting tumors (http://www.asianhealth secrets.com/black-cumin-seeds/).

Black cumin seed fixed oil contains appreciable quantities of unsaturated especially polyunsaturated fatty acids; constitute the bulk of oil ranging from 48-70%, while monounsaturated (18-29%) and saturated fatty acids (12-25%) are in lesser proportions (Nickavar *et al*., 2003; Cheikh-Rouhou *et al*., 2007). Besides better fatty acid profile, it also contains considerable quantities of tocopherols and allied bioactive compounds that are important in attenuating the overall antioxidant capabilities of the body (Valko *et al*., 2007).

Recent pharmacological investigations suggested its potential role, specifically for the amelioration of oxidative stress through free radical scavenging activity, the induction of apoptosis to cure various cancer lines, the reduction of blood glucose, and the prevention of complications from diabetes. It regulates hematological and serological aspects and can be effective in dyslipidemia and respiratory disorders. Moreover, its immune potentiating and immune modulating role brings balance to the immune system. Evidence is available supporting the utilization of Nigella sativa and its bioactive components in a daily diet for health improvement (Butt MS, Sultan MT., 2010).

the crude oil prepared from the seeds produce a variety of pharmacological actions such as antihistaminic (Chakravarty, 1993), diuretic and antihypertensive (Zaoui et al., 2000), hypoglycemic (Al-Hader et al., 1993), antioxytocic (Aqel and Shaheen, 1996), antinociceptive (Abdel-Fattah et al., 2000), respiratory stimulation (El-Tahir et al., 1993a), hematological (Enomoto et al., 2001) hepatoprotective (Daba and Abdel-Rahman, 1998) and immunopotentiating (Swamy and Tan, 2000) effects. The latter pharmacological properties

appear to be involved in the beneficial effects of N. sativa oil on headache, flatulence, blood homeostasis abnormalities, rheumatism and related inflammatory diseases (Boulos, 1983). Moreover, the seeds are believed to have carminative, stimulatory and diaphoretic properties and are used in the treatment of bronchial asthma and eczema (Boulos, 1983).

Black seed oil contains volatile oils including Nigellone and Thymochinone which are responsible for its antihistamine, antioxidant, anti-infective and broncho-dilating effects. The healing secrets of black seed oil are due to medicinal properties such as immune-stimulant, anti-bacterial, antifungal, anti-ulcerative, anti-inflammatory, antioxidant, anti-tumorous, antipyretic, hypoglycemic, immune-modulatory, antihypertensive, antidepressant, antispasmoic, hepato protective, anti-parasitic etc. (Javed, 2010).

3. MATERIALS AND METHODS

The experimental work was carried out in the Laboratory of Addis Ababa Institute of Technology School of Chemical and Bioengineering Laboratory (Analytical Chemistry Laboratory, Research Lab and Food lab) and Addis Abeba Collage of Natural Science Department of Chemistry. All the Materials that were used and the procedures followed are described below.

3.1. Raw material and equipments

3.1.1.Raw materials and Chemicals

Black cumin seed

The plant material that was black cumin seeds (nigella sativa) were collected from the local market in Addis Abeba (merkato) during November 2016 and explored further for their yield potential by using solvent extraction method.

Chemicals

There were different chemicals used during extraction and characterization. For extraction purpose Chemicals such as Ethanol (98%) and hexane (99.9%) were and for characterization purpose we used Glacial acetic acid, cyclohexane, potassium iodide, sodium thiosulphate, wijs solution, hydrochloric acid, potassium hydroxide (85 %), ethanol, sodium hydroxide (99%), phenolphthalein, filter paper, distilled water. The chemicals were purchased from different chemical sellers and some of them are obtained from School of Chemical and Bioengineering of Addis Ababa Institute of Technology.

3.1.2.Equipments

The equipments that used were

- Soxhlet extractor: used for extraction and recycle of the solvent.
- Thimble: used for putting the sample in soxhlet extractor
- Chiller: used to cool the water that used for condenser
- rotary evaporator: used for separate the solvent from the oil
- Condenser: used to cool the process and the solvent for further recycling
- Oven: used for drying the seed and determining the moisture content
- Flask and Beaker: used for putting the solvent for extraction process and collecting the oil

- Analytical Balance: used to determining the weight of that we want
- test tube: used for collecting of the oil
- GC-MS: used to determine the components that was found in the extracted oil
- FTIR: used to determine the functional groups that was found in the extracted oil

3.2. Experimental methods

3.2.1.Raw material preparation

Purification

The black cumin seeds were purchased from the local market; however the sample was not pure. The dusts, stones and other waste materials were removed manually and washed by water manually in order to remove other wastes.

Drying

After washing and removing all wastes from the seeds then we dried the seed by sun for one day. And also drying by oven was takes place in order to determine the moisture content of the seeds.

Size reduction

The dried black cumin seed have been grinded by a coffee grinder to a fine powder to be used directly after grinding. This is because the better yield can be obtained the particle size could be with the mesh size 0.5-0.25mm i.e. the seed was obtained in powder form (Henry, 1983; Alemu, 2016).

3.2.2.Extraction process using Ethanol and Hexane solvents

Soxhlet Extraction

Experimental work was conducted using soxhlet extractions. These soxhlet apparatus comprises a vertical glass cylindrical extraction tube that has both a siphon tube and a vapor tube, which is fitted at its upper end to a reflux condenser and at its lower end to a flask so that the solvent may be distilled from the flask into the condenser. From the condenser the solvent in liquid phase flows back into the cylindrical tube which holds the sample to be extracted in a porous thimble. When the solvent level rises to the top of the siphon tube the solvent, with the extracted materials, is siphoned over into the flask, to be distilled again, and thus start another cycle. After a sufficient period of time, all extractable matter in the sample is located in the flask, along with a major portion of the solvent, which is usually removed by

evaporation. Depending on the amount of solvent and its boiling point, evaporation as a process for removing the solvent can result in appreciable losses of volatile constituents of the sample.

Figure 3-1: Experimental setup on soxhlet extractor

Experimental Process description

The sample was placed in the thimble and was inserted in the center of the extractor. We used two different solvent for each experimental design factors so one of the solvent was pour into round bottom flask with respect to the ratio of the sample. The Soxhlet was heated at the boiling point of the solvents.This was allowed to continue for two, four, six and eight hours. The experiment was repeated by placing the same amount of the sample into the thimble again by varying seed: solvent ratio (w/v). Finally, the resulting mixture containing the oil was heated to separate and recover the solvent from the oil by using rotary evaporator. (Lawson *et al*., 2007). The extracted oil was measured and determined the physical and chemical properties. The recovery solvent was recycled and used for the next experiment.

The extraction was conducted in duplicate time, 20 gm. of black cumin seed with different seed solvent ratio:1:4, 1:6 and 1:8 and four different times: 2 hour; 4 hour ,6 hour and 8 hour, these value were selected based on previous research on similar seeds (Alemu, 2016).

3.3. Determination of percentage of oil yield

The percentage of extraction yield was using the formula below,

$$\text{percentage of extracted oil } X\frac{\text{mass of oil (in gm)}}{\text{mass of sample (in gm)}}*100\% \quad \textbf{.....3-1}$$

3.4. Characterization of the extracted oil

3.4.1.Physical properties of the extracted oil

Specific gravity

The specific density was calculated by first we measured the Density using density measuring machine then we divide the density of the sample that we got from the machine by density of the water.

$$\text{specific gravity } X\frac{\text{density of the oil}}{\text{density of the water}} \quad \textbf{.....3-2}$$

Kinematic viscosity of oil

First, a sample was heated at a temperature of 30^0C. The sample was fed to a sample holder of the Vibro Viscometer by measuring of 35 ml oil. Then the dynamic Viscosity of oil was displayed on the Vibro Viscometer screen when a sensor of the viscometer was immersed to the oil (Alemu; 2016).

Then the Kinematic Viscosity was calculated.

$$\text{kinematic viscosity of the oil(V) } X\frac{\mu}{\rho} \quad \textbf{.....3-3}$$

Where μ = Dynamic Viscosity and ρ = Density of oil

pH determination

pH determination can be done by Preparing 2 gm of the oil sample and placed in a 25 ml volume beaker and 13 ml of hot distilled water was added to the sample in the beaker and

stirred slowly. Then it was cooled in a cold water bath to 25^0C. The pH electrode was standardized with a buffer solution first and then the electrode immersed in to the sample and the pH was read and recorded (A.O.A.C Official Method of Analysis 960.19, 2000).

3.4.2.Chemical property of the extracted oil

Acid value

It is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of oil. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid.

The principle of acid value is determined by directly titrating the oil fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution.

AV is also an indicator for edibility of oil as well as their suitability for industrial use. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. The value is also expressed as percent of free fatty acids calculated as oleic acid. A low Acid Value (AV) means that an oil sample contains less free **fatty acid**s thus reducing its exposure to rancidification (Roger et al., 2010; Asuquo et al., 2012; Anderson-Foster et al., 2012).

Apparatus

- 250 Ml conical flasks

Reagent

- Ethyl alcohol
- Phenolphthalein
- Standard aqueous potassium hydroxide0.5N

Procedure

Weigh0.5 g accurately appropriate amount of the cooled oil sample in a 250 ml comical flask and add 50ml to 100ml of freshly neutralized hot ethyl alcohol and about one ml of phenolphalein indicator solution.Boil the mixture for about five minutes and titrate while hot against standard alkali solution shaking vigorously during the oil/fat taken for the estimation and the strength of the alkali used for titration shall be such that the volume of alkali required for the titration does not exceed 10ml.

The acid value was calculated as:

$$\text{Acid value (AV)} \; X56.1 * \frac{N * V}{W} \quad \text{.....3-4}$$

Where V = volume of standard potassium hydroxide (ml),

N = Normalityof potassium hydroxide solution

W= sample weight (in g)

The acidity is frequently expressed as free fatty acid for which calculation shall be:

$$\text{FFA as oleic acid } X\,28.2 * \frac{N * V}{W} \text{ percent by weight} \quad \textbf{.....3-5}$$

(Ref: I.S.I. Handbook of Food Analysis (Part XIII))

Saponification value

Saponification value is a measure of the alkali-reactive groups in fats and oil and is defined as the mg of KOH needed to saponify 1 g of oil (Shahidi, 2005). The saponification value of an oil increases with its lauric acid content. The la**uric acid**content and the Saponification value of oil serve as important parameters in determining the suitability of oil in soap making. Saponification value is also used in checking adulteration; a low value could suggest non-suitability of oil sample for industrial use.The principle is the oil sample saponified by refluxing with a known execss of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titeration of the excess potassium hydroxide with standard hydrochloric acid (HCl).

Apparatus

- 250ml capacity of conical flask with ground glass joints
- 1m long air condenser, or reflux condenser (65 cm minimum in length) to fit the flask
- Hot water bath or electric hot plate fitted with thermo stat

Reagents

- 0.5N of Alcoholic potassium hydroxide solution
- Phenolphthalein indicator solution
- Standard 0.5N of HCl

Procedure

Weigh the sample about 1.5 to 2.0 g and put in to a 250 ml Erlenmeyer flask. Pippete a 25ml of alcoholic potassium hydroxide solution in to the flask. Conduct a blank determination along with the sample. Connect the sample flask and the blank flask with air condensers, keep on the water bath, boil gently but steadily until saponification is complete, as indicated by the absence of any oily matter and appearance of clear solution. Clarity may be achieved with in one hour of boiling. Titrate the excess potassium hydroxide with 0.5N HCl, using about 1 ml phenolphthalein indicator.

(A.O.A.C official method of analysis 920.160, 2000).

$$\text{Saponification value (S.V)} \; X56.1 * \frac{N * (B - S)}{W} \quad \textbf{.....3-6}$$

Where: B = the volume in ml of standard HCl required for blank test;

S = the volume in ml of standard HCl required for the Sample;

N = Actual normality of the HCl used;

W = Weight of oil taken in gram.

Iodine value

Iodine is a simple chemical constant used to measure unsaturation or the average number of double bonds in an oil sample. It is defined as the number of grams of iodine that could be added to 100 g of oil (AOAC, 1990; Shahidi, 2005). The low iodine value for the oil indicates that the oil is rich in saturated **fatty acid**s, which ensures stability against oxidation and rancidification of foods prepared with the oil (Goh, 1994).

Apparatus:

500ml Erlenmeyer flask

Reagents:

- Cyclohexane
- Glacial acetic acid, free from ethanol
- Wijs Iodine monochloride solution
- Potassium Iodide (free from potassium iodate) – 10% solution prepared
- Distilled water
- Starch solution
- Sodium thiosulphate

Procedure

Weigh 0.3 to 0.4 g of sample in to 500ml conical flask with glass stopper, 15ml of equal amount mixture of cyclohexane and Glacial acetic acid had been added. Mix the content well and then pipette 25ml of Wij's solution and swirl for proper mixing and keep the flask in dark for 1hr in a dark area. Carry out blank simultaneously. After standing, add 15ml of potassium iodide solution, followed by 50ml of distilled water. Titrate liberated iodine with standardized sodium thiosulphate solution, using starch as indicator at the end until the blue color formed disappears after through shaking with the stopper on. Conduct a blank determination in the same manner as test sample but without oil.

The iodine value (I.V) is given by the expression:

$$\text{Iodine value (I.V)} \; X12.69 * \frac{N * (B - S)}{W} \quad \textbf{.....3-7}$$

Where: N = Normality of standard sodium thiosulphate used;

B = Volume in ml of sodium thiosulphate used for blank;

S= Volume in ml of sodium thiosulphate used for determination,

W = Mass of the sample.

(Ref: A.O.A.C. 17th edn, 2000, Official method 920.)

3.4.3.Composition analysis of the extracted oil

Gas Chromatography Mass Spectrometer (GC-MS)

Gas chromatography mas spectroscopy (GC-MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. GC-MS is highly effective and versatile analytical techniques with numerous scientific applications to cater the field of applied Sciences and Technology. It is a very useful for quality control, analytical research, impurity profiling and maintenance for human welfare and development.

In order for a compound to be analyzed by GC-MS it must be sufficiently volatile and thermally stable. In addition, functionalized compounds may require chemical modification (divertaisation), prior to analysis, to eliminate undesirable adsorption effects that would otherwise affect the quality of the data obtained. GC-MS analysis can be performed on liquids, gases or solids. For liquids and gases the sample is commonly directly injected into the GC. For solids, the analysis is carried out by solvent extraction, outgassing (desorption) or pyrolysis.

Desorption experiments are performed under helium flow at a controlled temperature between 40 – 300^0c, with analytes being collected on a cryogenic trap during desorption. Pyrolysis is another sampling technique for the analysis of the materials that cannot be directly injected into the GC-MS. By applying heat directly to the sample, the molecule can be broken down in a reproducible way. The smaller molecule are then introduced in to the GC and analyzed by GC-MS. By this method, probe temperatures of up to 1400^0c can be used. Many other sample preparation and sampling methods are available, such as

derivetization, static headspace analysis. Purge and trap, SPME (solid phase micro extraction) that have applications based on the sample type ad species of interest. (www.eag.com)

The sample solution is injected in to the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas (usually helium). The sample flows through the column and the compounds comprising the mixture of the interest are separated by virtue of their relative interaction with the coating of the column (stationary phase) and the carrier gas (mobile phase). The latter part of the column passes through a heated transfer line and ends at the entrance to ion source (figure) where compounds eluting from the column are converted to ions. (www.bris.ac.uk)

Identification of components

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver 8-Year 2005 library. The compound prediction is based on Dr. Duke's phytochemical and Ethno botanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. Interpretation of GC-MS was conducted using the database of NIST having more than 62.000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acid and nitro compounds (Muthulakshmi *et al*., 2012).

Fourier Transform Infrared (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is an effective analytical instrument that identifies chemical bonds in a molecule by producing an infrared absorptionspectrum. The spectra produce a profile of the sample, a distinctive molecular finger print that can be used to screen and scan samples for many different components. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. FTIR offers quantitative and qualitative analysis for organic and inorganic samples and detecting functional groups and characterizing covalent bonding information. (www.intertek.com)

FTIR spectrometers are widely used in organic synthesis, polymer science, petrochemical engineering, pharmaceutical industry and food analysis. In addition, since FTIR

spectrometers can be hyphenated to chromatography, the mechanism of chemical reactions and the detection of unstable substance can be investigated with such instruments. (https://chem.libretexts.org)

Functional groups are structural units within organic compounds defined by specific atom and bond arrangements. Infrared is a powerful identification tool for functional groups because of the similar absorption frequencies for those groups in different molecules. The actual frequency is affected by the environment, so the reference chart shows wide bonds rather than specific frequencies. The identification of functional groups is a cornerstone of IR spectroscopy and organic chemistry. (http://www.thermofisher.com)

3.4.4.Refining process of the extracted oil

Preparation of Clay

The clay sample that was used for bleaching purpose is bentonite clay and the clay was ground and then mixed with water. Impurities such as sand and stone were removed. To activate the clay, weigh 30gm of the clay and 150ml of 3M H2SO4 was added to the clay slurry and the mixture was boiled for 4hrs at about 100°C. The mixture was then washed with water to remove acid, dried and ground (Nkpa, N. N., *et al*, 1989)

Degumming

The extracted oil was degummed by the addition of boiling water. The mixture was stirred for 2 minutes and allowed to stand in the separating funnel. Thereafter, the aqueous layer was removed. The procedure was repeated to ensure removal of most gums (Nkpa, N. N., *et al*, 1989 and Carr R. A., 1976)

Neutralization

For the neutralization, about 60g of the degummed oil was poured into a beaker and heated to 80°C, after which 40ml of 0.1M NaOH was added and stirred to a uniform solution. Sodium chloride (about 10% of the weight of the oil) was added to help settle out the soap formed. This was transferred into a separating funnel and allowed to stand for 1h; the soap formed was separated from the oil. Hot water was added again and again to the oil solution until the soap remaining in solution was removed. The neutralized oil was then drawn off into beaker (Nkpa, N. N., et al, 1989 and Carr R. A., 1976)

Bleaching

50g of neutralized oil was poured into a beaker and heated to 90°C. Activated clay (4% by weight of oil) was added. The mixture was stirred continuously for 30 minutes. The temperature was allowed to rise to 110°C for another 30 minutes. The content was filtered hot in an over at 70°C (Carr R. A., 1976)

Deodorizing

The main uses of deodorizing process would be to remove some quantities of original odoriferous matter and trace chemicals that were used during neutralization process. So the procedure was poured the bleached oil in to a conical flask and heat at a temperature of 150 – 200^0c.

3.5. Design of the experiment

Full factorial design

Factorial design is used to investigate the effect of each factor. In a factorial experiment all the possible combination factor level would be tested and it would be possible to determine the effect of individual factors and to assess the effect of change of two or more variable at a time. The analysis was performed by utilizing design expert software using general full factorial design method. This method of experiment design helps to differentiate the significance of the main and the interaction factors. The software also used to develop the mathematical model that will describe the effect of the main and interaction factors on the response.

- **Factor:** 3 factors were investigated as mentioned earlier, those are: types of solvent, seed solvent ratio and time.
- **Factors levels:** for solvent = 2; for seed: solvent = 3 and for extraction time = 4 levels were considered.
- **Replicates:** Each independent experiment was repeated two times (k = 2).

Number of runs: For “m” level, “n” factors , and “k” replicate, the number of experimental runs that need to be performed is equal to k*n*m. But for our experiments the m value of each factors are different, so the experimental run will be = (m_1 * m_2 * m_3) * k.

These will be = (2 * 3 * 4) * 2 = 48 experimental runs were performed.

The factors and their levels

The levels that were selected for each factor are:

1. Types of solvent = Ethanol and Hexane
2. Seed solvent ratio = 1:4, 1:6 and 1:8
3. Extraction time (hour) = 2hr, 4hr, 6hr and 8hr.

Model equation

Finally, regression models were established for the dependent variables to fit the experimental data for the response using design expert 7.0.0 software.

4. RESULT AND DISCUSSION

4.1. Moisture content of the seed

The moisture content of the seed can be determined by 5g of the sample was placed in the oven that operates at temperature 105^0c and by interval of 2hr; we measured the sample until we got the same weight.

Table 4-1: Determination of the moisture content of the seed

Time (hr)	0	2	4	6	8	10
Weight (g)	5.012	4.928	4.853	4.781	4.753	4.752

The moisture content of the seed would be **= 5.47%**

4.2. Fixed oil yield by using Hexane and Ethanol solvents

From the table 4.2 and 4.3 the maximum yield of extraction in hexane solvent is 7.145 that is around 35.725% and obtained from with seed solvent ratio 1:8 and the time of 4 hour of extraction and the minimum yield was at the seed solvent ratio 1:4 and 2 hour of extraction time. Whereas the higher extraction yield using Ethanol solvent was 35.51% with the factor of the 1:6 of seed solvent ratio and with extraction time 6 hour and the minimum result was the seed solvent ratio 1:4 and 2 hours of extraction time that was similar factors that was used in hexane extraction. The extraction yield of 35.725 % using hexane solvent is lower that of the result of Gharby, et al; that afforded 37% from morocco seeds and also the result mentioned by Essam F. Al-Jumaily (Essam F. Al-Jumaily and Anwar Jalil Shihab Al-Jumaily, 2015) that they got 38% using a seed solvent ratio 1:7 for 8 hours. The result would be higher value than Abbas Ali et.al, where they got the yield of 32% using petroleum ether solvent (Abbas Ali et.al, 2011) and also with similar solvent Salma et.al, obtained 28.5% (Salma, C.R et.al., 2007).

Table 4-2: Extraction of black cumin oil using Hexane solvent

Hexane solvent						
No of run	Factors		Oil Yield (in gm.)			% yield
	Seed: Solvent (m/v)	Time (hr)	Rep 1	Rep 2	Average ± SD	
1	1 : 4	2	3.938	4.292	4.115 ± 0.250	20.575
2	1 : 4	4	4.501	5.091	4.796 ± 0.417	23.980
3	1 : 4	6	5.049	4.795	4.922 ± 0.180	24.610
4	1 :4	8	4.682	5.163	4.923 ± 0.340	24.615
5	1 : 6	2	5.181	4.824	5.003 ± 0.252	25.015
6	1 : 6	4	6.236	6.824	6.530 ± 0.416	32.650
7	1 : 6	6	6.615	7.139	6.877 ± 0.371	34.385
8	1 : 6	8	7.141	6.623	6.882 ± 0.366	34.41
9	1 : 8	2	5.172	5.846	5.509 ± 0.477	27.545
10	1 : 8	4	7.536	6.754	7.145 ± 0.553	35.725
11	1 : 8	6	6.735	7.358	7.047 ± 0.441	35.235
12	1 : 8	8	6.924	7.121	7.023 ± 0.139	35.115

***Rep = Replication**

***SD = Standard Deviation**

Table 4-3: Extraction black cumin oil yield using ethanol solvent

Ethanol solvent						
No of run	Factors		Oil Yield (in gm.)			% yield
	Seed: Solvent (m/v)	Time (hr)	Rep 1	Rep 2	Average ± SD	
1	1 : 4	2	4.275	4.849	4.562 ± 0.406	22.81
2	1 : 4	4	5.144	4.698	4.921 ± 0.315	24.605
3	1 : 4	6	5.251	5.023	5.137 ± 0.161	25.685
4	1 :4	8	4.994	5.218	5.106 ± 0.158	25.530
5	1 : 6	2	6.072	6.238	6.155 ± 0.117	30.775
6	1 : 6	4	6.291	6.521	6.406 ± 0.163	32.03
7	1 : 6	6	6.906	7.298	7.102 ± 0.277	35.51
8	1 : 6	8	6.511	6.975	6.743 ± 0.328	33.715
9	1 : 8	2	6.255	6.387	6.321 ± 0.093	31.605
10	1 : 8	4	6.533	6.835	6.684 ± 0.214	33.420
11	1 : 8	6	6.925	6.964	6.945 ± 0.028	34.725
12	1 : 8	8	6.637	6.903	6.770 ± 0.188	33.850

***Rep = Replication**

***SD = Standard Deviation**

4.3. Effect of individual factors on the yield of Fixed oil

4.3.1.Effect of seed solvent ratio on the yield of Fixed oil

The effects of seed to solvent ratio on the oil yield have been shown in the figure 4.1 and figure 4.2 using hexane and ethanol solvent respectively. As we seen from the figure that was done by design expert software, that determine the effect of seed solvent ratio on the oil yield by making the time factor is constant, that takes actual time of 5 hr for both graphs, and the yield of the oil increases where the seed to solvent ratio increases and goes to maximum limit, then it seems like a straight line where the seed solvent ratio increases in figure 4.7. The maximum oil yield using hexane solvent is obtained by the seed solvent ratio of 1:8.

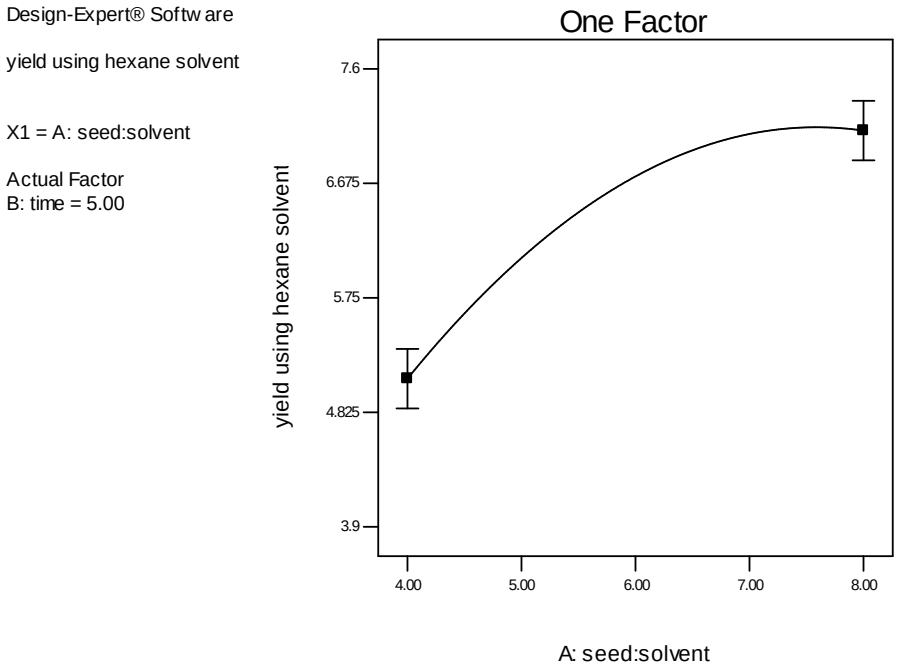


Figure 4-1: Effect of seed solvent ratio on the yield extraction using hexane solvent

Similarly the effect of the seed solvent ratio on the oil yield using ethanol solvent is, when the seed solvent ratio is increases; the oil yield will be steadily increases significantly and then decreases with respect to the seed solvent ratio. This is because the effect of the seed solvent ratio on the yield of the oil is low when the ratio increases. From the experiment graph the maximum yield that we obtained using ethanol solvent with the seed solvent ratio 1:6. This implies that the 1:8 seed solvent ratio gets fewer yields than that of the 1:6.

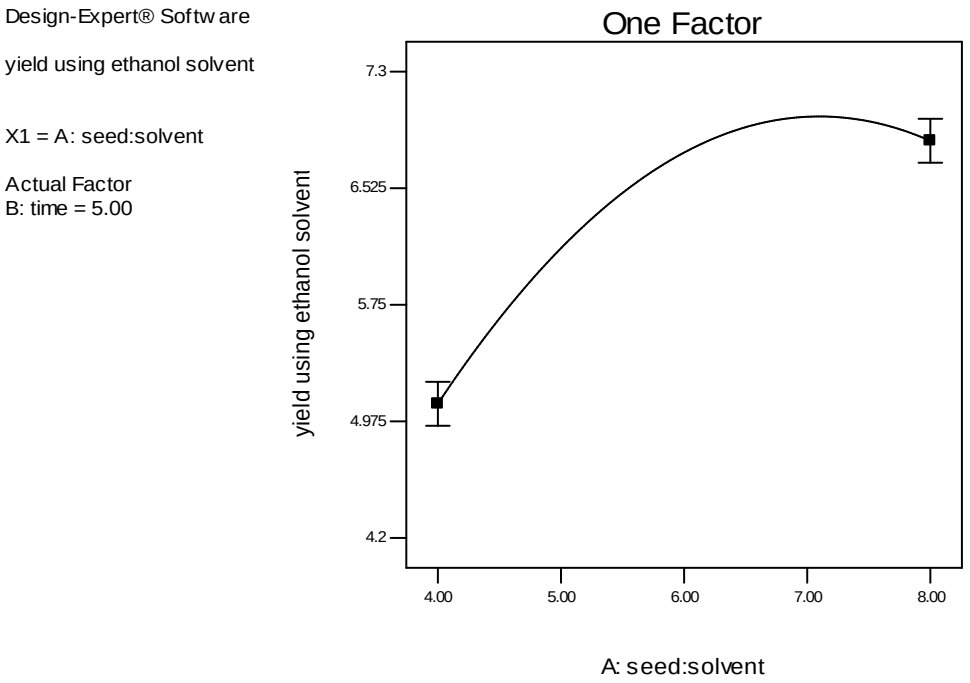


Figure 4-2: Effect of seed solvent ratio on ethanol solvent extraction

4.3.2.Effect of the extraction time on the yield of Fixed oil

As we can see the graph the percentage oil yield was directly related to extraction time at some extent and then decreases a little, which means the uses of excess time of extraction there is no any effect on the extraction yield. Figure 4.3 and figure 4.4 shows that the effect of extraction time on the oil yield using hexane solvent and ethanol solvent respectively. The maximum oil yield that we got from the experiment is at the extraction time of 4hr for hexane used as a solvent and similarly for using the ethanol solvent the maximum yield could be a time of 6hr extraction.

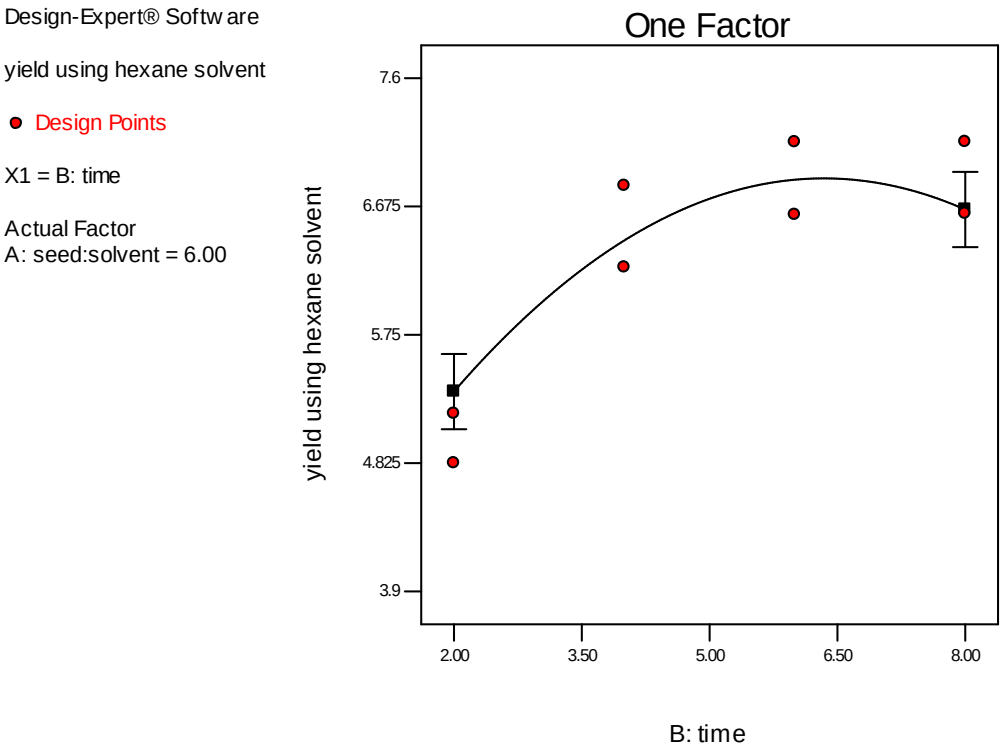


Figure 4-3: The effect of time on extraction yield using hexane solvent

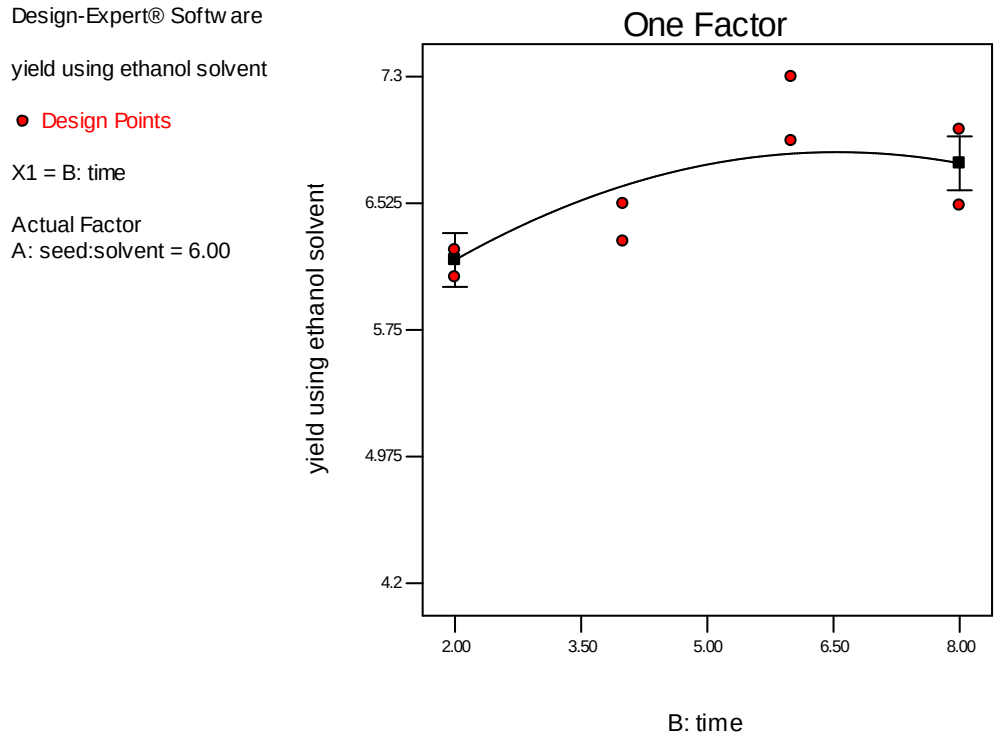


Figure 4-4: The effect of time on extraction yield using Ethanol solvent

4.3.3.Effect of solvent type on the yield of Fixed oil

The effect of the types of solvent has a great effect on the extraction of the oil. Different researchers are study the effect of the solvent in the yield, Phsyco - chemical properties and the constituents of different seeds oil. In this paper we use two solvents namely; hexane and ethanol and we try to analyze the effect of the two solvents in the yield of black cumin fixed oil. Figure 4.5 shows the effect of both solvents in the yield of the oil using the reference of the extraction time and at low time the ethanol solvent gave a higher yield than the hexane solvent and as a time increases the higher yield would be got from the hexane solvent.

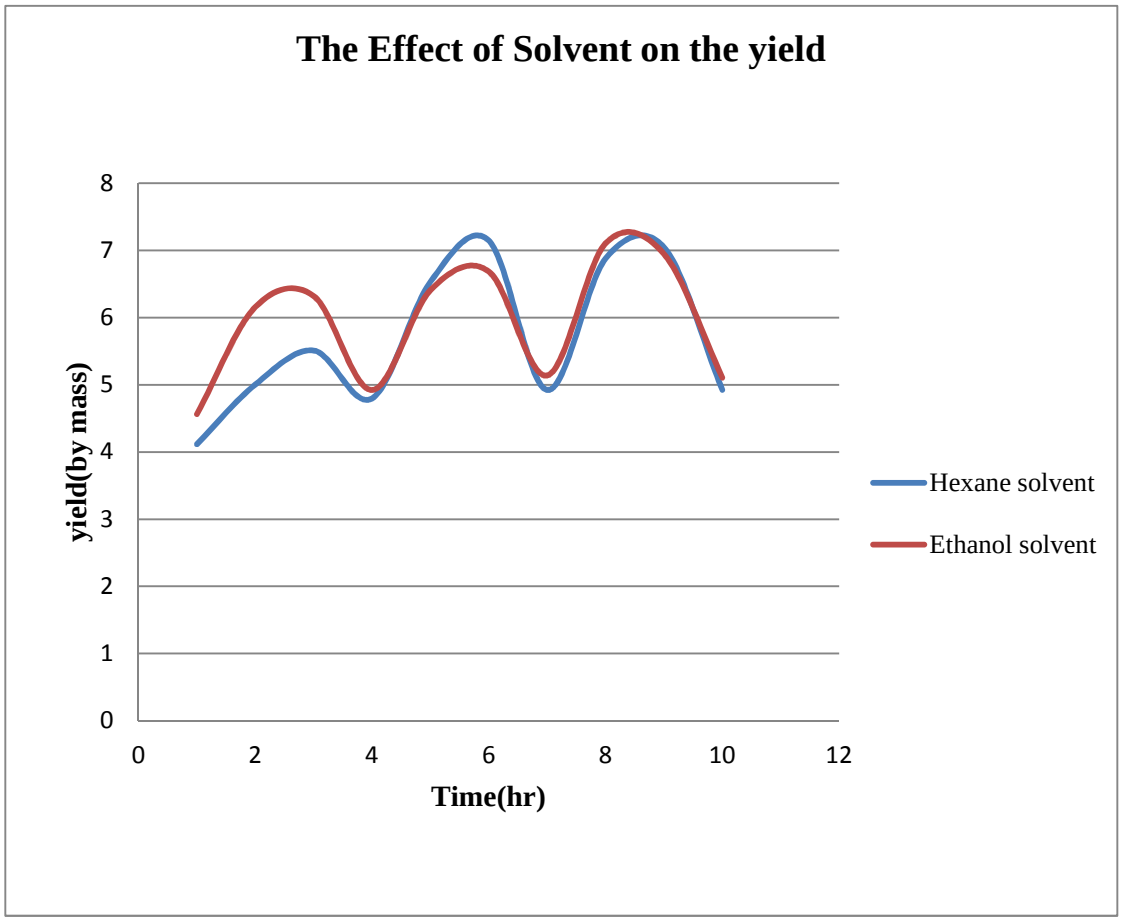


Figure 4-5: Effect of the solvent type on extraction yield

4.4. Optimization of the extraction of black cumin Fixed oil

Numerical optimization

Numerical optimization will search the design space, using the models that created in the analysis, to find factor settings that meet the goals we define. On the criteria we were define the goals for each response. Finally, the software will generate a list of potential factor settings that provide responses that meet the criteria that defined. To check the desirability

and if we want to maximize the response that is the yield, we would select the goal would be “maximize” and the upper limit would be the higher yield and the lower limit would be the minimum yield that we got from when we done the experiment in criteria section.

The desirability function is an objective function that is used for optimization of multiple quality characteristic problems. And transform the estimated response to a scale free value. The desirability will be ranging from 0 to 1 and completely dependent on closeness to the lower and upper limits. If the desirability value shows 1 it represents the ideal case; 0 indicates the one or more responses are being outside their acceptable limits. Depending on whether a particular response is to be maximized or minimized or assigned to a target value, various desirability functions can be used. Table 4.4 shows the criteria that were set for optimization run. The upper limit and the lower limit of the main factors that was set in the software would be the maximum and the minimum level of each factor respectively. That is for seed solvent ratio the lower limit 1 : 4 and the upper limit was 1 : 8 and similarly for extraction time the upper limit 8hr and the lower limit was 2 hr. The goal of the response would be set to “in range” for the two factors and set to “maximize” for the two responses. The “upper limit” and the “lower limit” of the two responses would be set to the maximum and the minimum yield (“Upper limit” = 7.558; “Lower limit” = 3.938 for yield using hexane solvent and 7.298; 4.275 for the upper and lower limit respectively of the yield using ethanol solvent). There was selected one solution that got from the numerical optimization experiment with respect to that we need and the result is the seed solvent ratio 7.38, with time of 6.54hr, the yield become 7.31892 and 7.05573 using hexane and ethanol solvent respectively.

The optimal parameter conditions are those with factor settings with maximum total desirability (TD).The objective function simultaneous is a geometric mean of all transformed responses. Based on general factorial desirability optimization technique, design expert software is used to evaluate the combination. Both the yield of different solvents has been optimized using developed models. The optimal solution is to evaluate the input parameters in the process range for maximizing the yield.

Table 4-4: Constraints of solution for numerical optimization

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
seed:solvent	is in range	1 : 4	1 : 8	1	1	3
Time	is in range	2	8	1	1	3
yield using hexane solvent	Maximize	3.938	7.536	1	1	3
yield using ethanol solvent	Maximize	4.2758	7.298	1	1	3

Solutions

Number	Seed: Solvent	Time	Yield using hexane solvent	Yield using ethanol solvent	desirability	
1	7.32	6.52	7.2952	7.06156	0.927	Selected

A set of thirty optimal combinations for solution is derived for the design space constraints for the yield using Design Expert software. The set of conditions with the highest desirability value is considered as optimum combination for the required responses. The optimal combinations with higher desirability functions are given in table 4.4. The values of optimum parameters combination and predicted values of responses have been tabulated in tables 4.5 and 4.6 respectively.

Table 4-5: Optimum value of the oil yield

Process parameter	Goal	Optimum value
Seed to solvent ratio (m/v)	Is in range	1:7.32
Extraction time (hr)	Is in range	6.52

Table 4-6: Predicted and observed value of yield

Response	goal	predicted	Observed	Error (%)
Yield using hexane solvent	maximize	7.2952	7.536	3.3
Yield using ethanol solvent	maximize	7.06162	7.298	3.35

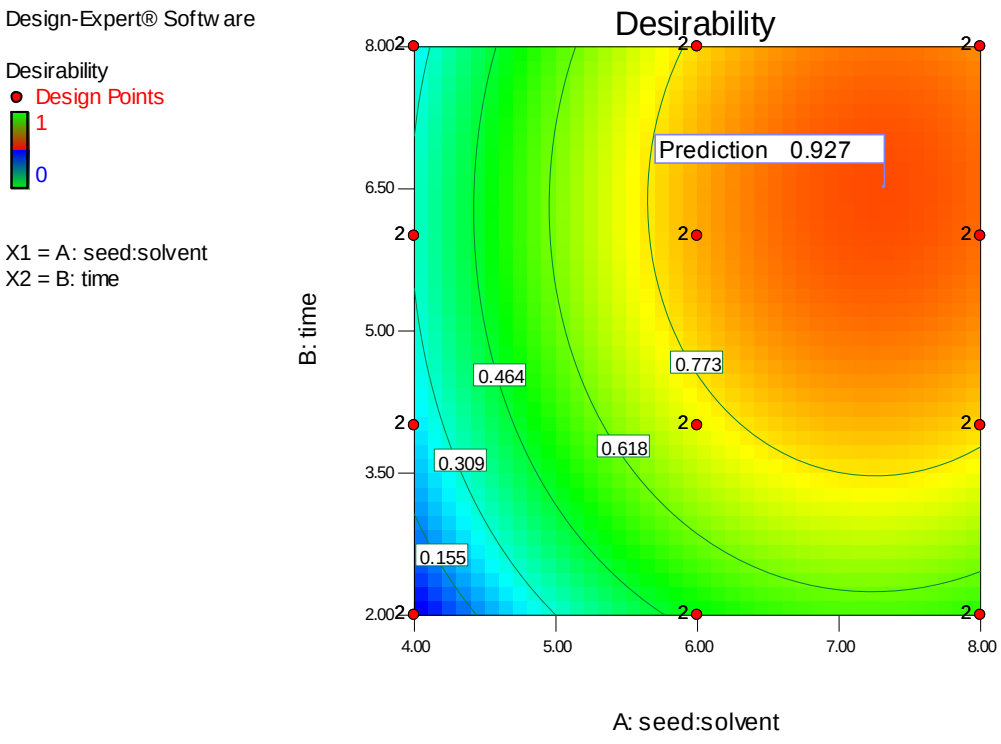


Figure 4-6: The desirability prediction of the optimization yield

From the analysis, which is described in the table 4.6, it is observed that the error is small. Obviously, this shows perfect reproducibility of the experiment conclusions. The ramp

functions graph and bar graph (see in the appendix B_4) shows the desirability for output responses. The dot on each ramp indicates the factor setting (or) response prediction for that response characteristic. The height of the dot reflects how much desirable it is. A linear ramp functions is created between lower value and the required value (or) the higher value and the required value as the weight for each parameter is set equal to 1. The bar graph indicates the overall desirability of the responses. The optimal region values have the overall desirability value of 0.927 indicating the closeness to the target response.

4.5. Characterization of the extract black cumin Fixed oil

As we discussed in the above, in optimization section; the seed solvent ratio and the extraction time has a great effect in the oil yield in both hexane and ethanol solvent. The optimum process parameter value that is described in the table 4.11 to get the optimum result that is 7.2952gm (36.5%) for hexane solvent and 7.06gm (35.3%) for ethanol solvent; would be the seed to solvent ratio of **1:7.32** and the extraction time of **6.52hr** in both solvents type.

4.5.1. Physical properties of the extracted Fixed oil

Specific gravity

The density of the oil was measured by a density measuring machine. After injecting the sample in to the machine the machine reads the density, specific density and the working temperature.

Therefore, the density of the oil would be:

Density of HSO = $0.9199 g/cm^3$

Density of ESO = $0.9215 g/cm^3$

The specific gravity would be:

$$\text{Sp.gravity } X\frac{\text{density of oil}}{\text{density of water}}$$

$$\text{Sp.gravity HSO } X\frac{0.9199\ g/cm3}{1\,g/cm3} = \mathbf{0.9199}$$

$$\text{Sp.gravity ESO } X\frac{0.9215\ g/cm3}{1\,g/cm3} = \mathbf{0.9215}$$

Where; HSO = Hexane Solvent Oil; ESO = Ethanol Solvent Oil

As we can see described in the above the specific gravity of the black seed oil extracted from hexane and ethanol solvents are 0.9199 and 0.9215. The result that we got from this experiment would be less than M. Kamal E. Youssef that was 0.9424 and also a good agreement with black seed oil suppliers such as aromatic ltd (www.segevfood.com) and natural sourcing (https://www.naturalsourcing.com)

The specific gravity of the extracted oil estimated in the above also higher than the value 0.9071 at 25^0C and 0.8840 at 30^0C that were reported by Mariod et al (Mariod et al, 2006).

Viscosity of the oil

The Dynamic viscosity of the oil that was read from the viscometer at temperature of 28^0C was 64.53mPa.sec for HSO and 67.25mPas.sec for ESO and the Kinematic Viscosity was calculated using the formula that describe in the equation 3.3 and the result would be:

$$\text{Dynamic viscosity of HSO} = 64.53\text{mPa.sec} = 64.53*10^{-3}\ \text{kg/ms}$$

$$\text{Kinematic viscosity of HSO} = 70.15*10^{-3}\ \text{m}^2/s,$$

$$\text{Dynamic viscosity of ESO} = 67.25\text{mPa.sec} = 67.25*10^{-3}\ \text{kg/ms}$$

$$\text{Kinematic viscosity of ESO} = 72.98*10^{-3}\ \text{m}^2/s$$

pH value of the oil

The pH value of the yield oil was determined by using the pH meter. We try to determine the pH value with three replications as shown in the table 4.7.

Table 4-7: The pH value of the extracted oil

Oil type	Rep 1	Rep 2	Rep 3	Average ± SD
P^H of HSO	6.27	6.24	6.33	6.28 ± 0.046
P^H of ESO	5.96	6.01	5.97	5.98 ± 0.026

As we can see the table the pH vale of the oil using the two different solvent would be 6.28 ± 0.036 and 5.98 ± 0.026 with hexane and ethanol solvent respectively. The pH value is greater than 4.16 that were described in mountain herbs .com that produced in Egypt.

4.5.2. Chemical properties of the extracted Fixed oil

Iodine value

The iodine value of oil is the number of gram of iodine absorbed by 100g of oil, when determined by using Wijs solutions. It is used to determine the amount of unsaturation fatty acids. This unsaturation is in the form of double bonds, which react with iodine compounds. The higher the iodine number, the more C=C bonds are present in the fat. The iodine vale of the extracted oil would be shown in the table 4.8 below. The volume (in ml) of standard sodium thiosulphate solution required for the blank determination for iodine value would be 40.1ml. The iodine value of the extracted oil would be an average of 96.3 ± 2.605 and 74.346 ± 2.060 using hexane and ethanol solvent respectively. This result would be less than the Mervat El-Demery (Mervat El-Demery and Msotafa A. Owon et.,al); that was reported the iodine value of **156 g I_2/100g oil.** It was also lower than 114 and 119 that were reported by Nawal.H.Al.Bahtiti and Salma *et al.*

Table 4-8: Iodine value of the extracted oil

FIXED OIL USING HEXANE SOLVENT				
Run	Rep 1	Rep 2	Rep 3	Average ± SD
Mass of sample (gm)	0.392	0.365	0.377	0.378 ± 0.014
Volume of $Na_2S_2O_3$ (ml)	11.2	11.7	11.4	11.43 ± 0.252
Iodine value(as g I_2/ 100 g oil)	93.556	98.739	96.606	**96.30 ± 2.605**
FIXED OIL USING ETHANOL SOLVENT				
Run	Rep 1	Rep 2	Rep 3	Average ± SD
Mass of sample (gm)	0.346	0.371	0.385	0.367 ± 0.02
Volume of $Na_2S_2O_3$ (ml)	19.3	18.3	18.2	18.6 ± 0.608
Iodine value (as g I_2/ 100 g oil)	76.287	74.567	72.185	**74.346 ± 2.060**

Saponification value

As we know saponification value represents the number of milligrams of potassium hydroxide required to saponify 1g of fat under the conditions specified. To calculate the saponification value, using the formula that we would described in equation 6 in materials and methods section; and we used the volume in ml of standard hydrochloric acid required for the blank experiments would be 26 ml. The saponification value of the black cumin oil using the two solvent could described in the table below, and the result would be 202.01 ± 2.657 and 197.6233 ± 2.09 using hexane and ethanol solvent respectively. These implies, the saponification value of the two samples of black cumin seed oil as estimated indicates the presence of higher proportion of higher fatty acids. These numbers are actual saponification value (SAP) values expressed in the number of mg of KOH required to saponify 1 g of oil/fat.

Table 4-9: Saponification value of the extracted oil

FIXED OIL USING HEXANE SOLVENT				
	Rep 1	Rep 2	Rep 3	Average ± SD
Weight of sample	1.782	1.924	1.886	1.864 ± 0.073
B (ml)	26	26	26	26
S (ml)	13.4	12.1	12.7	12.73 ± 0.65
Saponification value	**198.33**	**202.65**	**197.81**	**202.01 ± 2.657**
FIXED OIL USING ETHANOL SOLVENT				
	Rep 1	Rep 2	Rep 3	Average ± SD
Weight of sample	1.752	1.674	1.812	1.746 ± 0.07
B	26	26	26	26
S	13.8	14.1	13.2	13.7 ± 0.46
Saponification value	**195.32**	**199.4**	**198.15**	**197.6233 ± 2.09**

When reading a certificate of analysis of an oil/fat from different websites, the saponification Value will be listed in this way. Dividing the SAP value by 1000 gives us a ratio of KOH to Oil in the same units (mg). So the ratio of KOH to oil of HSO = 0.202 and ESO= 0.197 g of KOH per g of oil.

The investigated saponification value results of the two samples was greater than 172.56 mg KOH/ g oil that was described in Mervat El-Demery (Mervat El-Demery and Msotafa A. Owon et.,al), but it was lower than the values 211-218 mentioned by Salma et al.(Salma et al., 2013) and 203 mentioned by Atta (Atta, 2003) and Nawal.H.Al.Bahtiti for the same seed oil.

Acid value

The acid value of the oil expresses the number of mg of potassium hydroxide required to neutralize the free acids in 1.0g of the substance. In order to determine the acid value of our extracted oil we used 0.5g of the sample, 0.5N of potassium hydroxide (0.5N KOH) for the titration and 75 ml hot ethyl alcohol. Table 4.10 shows that the acid value of the extracted oil with the two solvents.

Table 4-10: Acid value of the extracted oil

FIXED OIL USING HEXANE SOLVENT				
Run	Rep 1	Rep 2	Rep 3	Average ± SD
Volume of KOH (ml)	2.3	2.2	2.7	2.4 ± 0.265
Acid value	0.129	0.123	0.151	**0.134 ± 0.015**
FFA (% by weight)	0.065	0.062	0.076	**0.068 ± 0.007**
FIXED OIL USING ETHANOL SOLVENT				
Run	Rep 1	Rep 2	Rep 3	Average ± SD
Volume of KOH (ml)	1.9	1.4	1.8	1.7 ± 0.265
Acid value	0.107	0.078	0.101	**0.095 ± 0.015**
FFA (% by weight)	0.054	0.039	0.051	**0.048 ± 0.008**

From the table, the investigation of acid value of the two samples would be (0.134 ± 0.015 and 0.048 ± 0.008 for HSO and ESO respectively,) lower than 2.80 that was mentioned by Mervat El-Demery (Mervat El-Demery and Msotafa A. Owon et.,al), and also the FFA value would be much lower than 12% that was described by Nawal.H.Al.Bahtitiand 0.82% that was mentioned M. Kamal E. Youssef et.al, (M. Kamal E. Youssef, et al, 2013).

4.5.3. Composition analysis of the extracted Fixed oil

Gas Chromatography Mass Spectroscopy (GC-MS)

The result of each the component that appear in the sample was determined by Gas Chromatography – Mass Spectroscopy (GC – MS) by injecting 1⇑L of the solution . The specification of the GC - MS was Agilent Technology 7820A gas chromatograph with a split detector and coupled with Mass Spectrometer Detector (MSD), 5977 Mass Spectrometer Detector (MSD) and 0.25mm * 30mm fused – silica capillary column bonded with 0.25 ⇑m layer of phase G16.

The chromatograph is programmed to maintain the column temperature at 60^0C for about 2 min after injection, then toincrease the temperature at the rate of 5^0C/min to 325/350^0C, and finally to maintain this temperature for 5 min. The injection port temperature is maintained at about 350^0C. The carrier gas is helium with a linear velocity of about 50 cm/s. Table 4.18 shows that the GC-MS analysis of the sample.

The GC – MS analysis of the ethanol extract oil was not carried out because of there was no any institute or company has willing to done the analysis. The reason was there was no any standard value and reagents to facilitate the machine and so what shall done was, read the components from the literature that was done by K. Nivetha and G. prasanna (K. Nivetha and G. prasanna, 2016) by similar process.

The procedure is 2µl of the ethanol extract of *Nigella sativa* was employed for GC-MS analysis. The Clarus 436 GC Bruker used in the analysis employed a fused silica column BR-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m × 0.25mm ID × 0.25µm 2µl df and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The sample extract injected into the instrument which was detected by the Turbo gold mass detector (perkinelmer) with the aid of the software MS work station 8. During the 36th minute of GC extraction process, the oven was maintained at a temperature of 110º C with 2 minutes

holding. The injector temperature was set at 250ºC (Mass Analyzer). The different parameters involved in the operation of the Clarus 436 MS Bruker were also standardized (Inlet line temperature: 200ºC). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes (Srinivasan *etal*., 2013).

The compounds were identified through mass spectrometry attached with GC with respect to their peak area and retention time. Totally 23 compounds were identified on hexane extracts namely 1,3,5-Cycloheptatriene,3,7,7-trimethyl (1.3%), Acetamide,N-(aminocarbonyl)-2-chloro (0.44%), Thymol (2.88%), Methylpent -4-enylamine (1.12%), Phenol, 2-methyl-5-(1-methylethyl) (2.68%), Tetradecane (2.4%), Ethylamine, 2-(adamantan-1-yl)-1-methyl (0.84%), 1,2,5-oxadiazol-3-carboxamide, 4,4-azobis-, 2,2-dioxide (0.67%), .beta.-alanine, N-methyl-, ethyl ester (0.74), Pentadecane (3.77%), 2-Butanamine, 3-methyl- (1.02%), Amphetamine (0.7%), Indole, 5-Methoxy-3-(2-methylamino)ethyl (2.28%), p-Hydroxynorephedrine (1.78%), Tocainide (4.72%), 2-propenoic acid, tridecyl ester (4.28%), 1,3-propanediamine, N-(2-aminoethyl) (1.88%), Tenamfetamine (1.88%), Hexadecanoic acid, methyl ester (12.94%), Phenethylamine, p,.alpha.-dimethyl (1.52%), 9,12-Octadecadienoic acid (Z,Z)-,Methyl ester (23.11%), 6-Octadecenoic acid, methyl ester, (z)- (21.782%) and 1-propanamine, N,2-dimethyl- (2.07%).

Whereas on ethanol extract totally 28 compounds were identified namely 5-Hydroxymethylfurfural (0.02%), Thymoquinone (0.03%), Thymol (0.06%), 2-Isopropylidene-5-methylhex-4-enal (0.02%), Longifolene (0.03%), Phenol, 3-(1,1-dimethylethyl)-4- methoxy- (0.02%), P-tert-Butyl catechol (0.52%), Tetradecanoic acid (0.18%), Tetradecanoic acid, ethyl ester (0.15%), Hexadecanoic acid, methyl ester (0.14%), n-Hexadecanoic acid (29.42%), Hexadecanoic acid, ethyl ester (11.31%), 9,12-Octadecadienoic acid, methyl ester (1.03%), Oleic Acid (0.65%), 9,12-Octadecadienoic acid (Z,Z)- (10.39%), Eicosanoic acid (13.39%), cis-13,16- Docasadienoic acid (2.55%), cis-11,14-Eicosadienoic acid, methyl ester (3.93%), methyl 19 hexacosenoate (1.06%), Chelestan-3-ol, 2-methylene-, (3β,5α)- (0.08%), 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy) methyl] ethyl ester, (Z,Z,Z)-(0.96%), Palmitic acid β-monoglyceride (2.92%), 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- (0.79%),9,12-Octadecadienoic acid(z-z)-,2-hydroxy 1-(hydroxymethyl)ethyl ester (19.11%), Supraene (0.08%), Campesterol (0.13%), Stigmasterol (0.14%), β-Sitsosterol (0.40%).

Table 4-11: GC-MS analysis of hexane extract sample

No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1	6.855	1,3,5-Cycloheptatriene,3,7,7-trimethyl-	$C_{10}H_{14}$	134.22	1.30
2	8.034	Acetamide,N-(aminocarbonyl)-2-chloro	$C_3H_5ClN_2O_2$	136.535	0.44
3	10.914	Thymol	$C_{10}H_{14}O$	150.22	2.88
4	10.991	Methylpent -4-enylamine	$C_6H_{13}n$	99.174	1.12
5	11.066	Phenol, 2-methyl-5-(1-methylethyl)	$C_{10}H_{14}O$	150.217	2.68
6	12.352	Tetradecane	$C_{14}H_{30}$	198.39	2.40
7	12.826	Ethylamine, 2-(adamantan-1-yl)-1-methyl	$C_{21}H_{28}N_2$	308.46	0.84
8	12.945	1,2,5-oxadiazol-3-carboxamide, 4,4-azobis-, 2,2-dioxide			0.67
9	13.524	.beta.-alanine, N-methyl-, ethyl ester	$C_{10}H_{19}NO_4$	217.267	0.74
10	13.632	Pentadecane	$C_{15}H_{32}$	212.41	3.77
11	13.848	2-Butanamine, 3-methyl-	$C_5H_{13}n$	87.1634	1.0213
12	14.000	Amphetamine	$C_9H_{13}N$	135.21	0.70
13	14.089	Indole,5-Methoxy-3-(2methylamino)ethyl	$C_{12}H_{16}N_2O$	204.373	2.28
14	14.844	p-Hydroxynorephedrine	$C_9H_{13}NO_2$	167.21	1.78
15	14.906	Tocainide	$C_{11}H_{16}N_2O$	192.258	4.72
16	15.932	2-propenoic acid, tridecyl ester	$C_{16}H_{30}O_2$	254.414	4.28
17	16.291	1,3-propanediamine, N-(2-aminoethyl)	$C_5H_{15}N_3$	117.196	1.88
18	18.755	Tenamfetamine	$C_{10}H_{13}NO_2$	179.22	1.59
19	18.899	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	12.94
20	19.151	Phenethylamine, p,.alpha.-dimethyl	$C_{10}H_{15}N$	149.23	1.52
21	21.684	9,12-Octadecadienoic acid (Z,Z)-,Methyl ester	$C_{19}H_{34}O_2$	294.472	23.11
22	21.782	6-Octadecenoic acid, methyl ester, (z)-	$C_{19}H_{36}O_2$	296.49	25.27
23	22.229	1-propanamine, N,2-dimethyl-	$C_5H_{13}N$	87.16	2.07

Table 4-12: GC-MS analysis of Ethanol extract sample

No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1	5.82	5Hydroxy methyl furfural	$C_6H_6O_3$	126	0.02
2	6.27	Thymoquinone	$C_{10}H_{12}O_2$	164	0.03
3	7.05	Thymol	$C_{10}H_{14}O$	150	0.06
4	8.23	2-Isopropylidene-5-methylhex-4-enal	$C_{10}H_{16}O$	152	0.02
5	8.84	Longifolene	$C_{15}H_{24}$	204	0.03
6	9.63	Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	$C_{11}H_{16}O_2$	180	0.02
7	10.65	p-tert-Butyl catechol	$C_{10}H_{14}O_2$	166	0.52
8	13.07	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.18
9	13.42	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.15
10	15.09	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.14
11	15.91	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	29.42
12	16.05	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	11.31
13	17.41	9,12-Octadecadienoic acid, methyl Ester	$C_{19}H_{34}O_2$	294	1.03
14	17.51	Oleic Acid	$C_{18}H_{34}O_2$	282	0.65
15	18.68	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	10.39
16	18.95	Eicosanoic acid	$C_{20}H_{40}O_2$	312	13.39
17	21.11	cis-13,16-Docasadienoic acid	$C_{22}H_{40}O_2$	336	2.55
18	21.38	cis-11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	3.93
19	21.84	Methyl 19-hexacosenoate	$C_{27}H_{52}O_2$	408	1.06
20	23.02	C21H38O2 C22H40O2 C22H40O2	$C_{28}H_{48}O$	400	0.80
21.	23.31	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	$C_{25}H_{40}O_6$	436	0.96
22.	23.60	Palmitic acid β-monoglyceride	$C_{19}H_{38}O_4$	330	2.92
23		9,12-Octadecadienoic acid(z-z)-,2-hydroxy 1-(hydroxymethyl)ethyl ester	$C_{19}H_{34}O_2$	294.47	19.11
24	-	Supraene	$C_{30}H_{50}$	410.725	0.08
25	-	Campesterol	$C_{28}H_{48}O$	400.691	0.13
26	-	Stigmasterol	$C_{29}H_{48}O$	412.69	0.14
27	-	β-Sitsosterol	$C_{29}H_{50}O$	414.71	0.40

Among the compounds, major peak values were obtained for the compounds in hexane extract such as 9, 12-Octadecadienoic acid (Z, Z)-, Methyl ester (23.11%), 6-Octadecenoic acid, methyl ester, (z) - (21.782%) and Hexadecanoic acid, methyl ester (12.94%) and for ethanol extract Among the 28 compounds, major peak values were obtained for the compounds such as n-Hexadecanoic acid (29.42%), 9.12- Otadecadienoic acid (z,z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (19.11%), Eicosanoic acid (13.39%), Hexadecanoic acid, ethyl ester (11.31%), 9,12-Octadecadienoic acid (Z,Z)- (10.39%).

The identified compounds possess many biological properties that are described in the table (table 4.13) that is found in the two solvent extracts crude oils. All of the compounds that are described in the above are found in ethanol extracts.

4.5.4. FT-IR analysis of the extracted oil

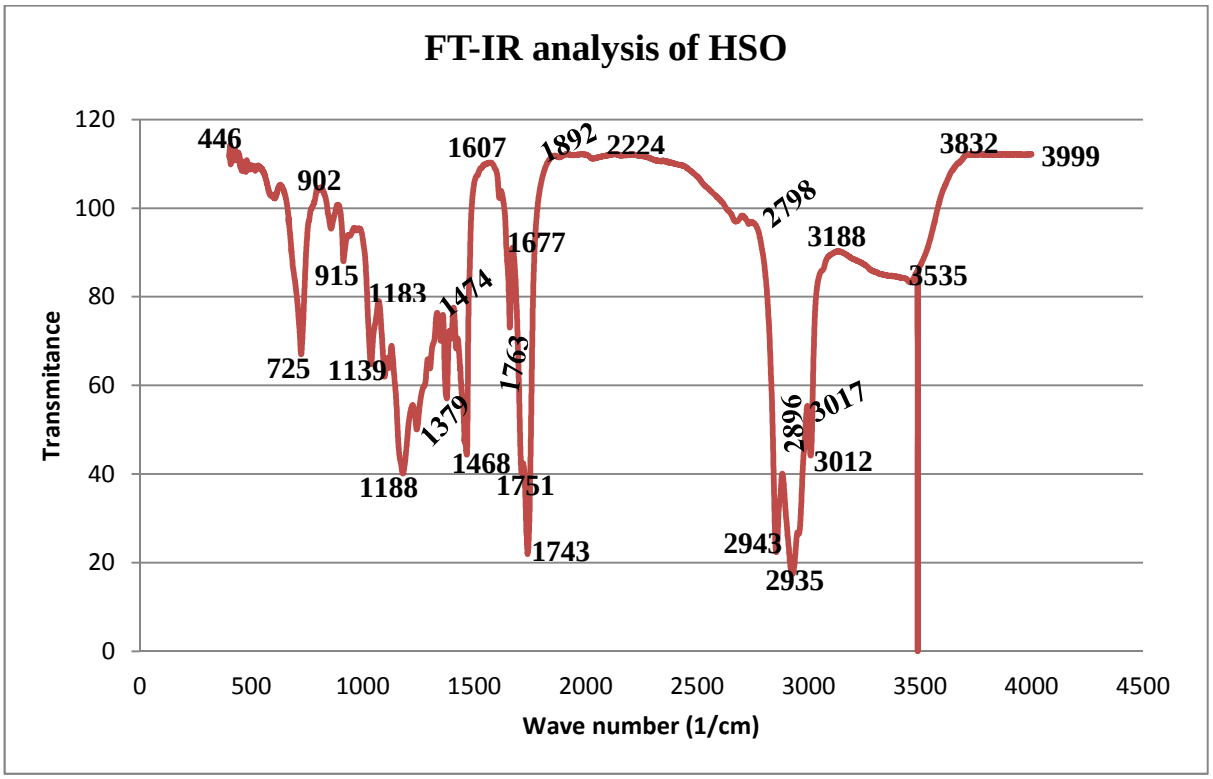


Figure 4-7: FT-IR analysis of the extracted oil using Hexane solvent

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a

molecule can be determined. Figure 4.7 and 4.8 exhibits FTIR spectra of black cumin seed oil using hexane and ethanol solvents at mid-infrared region range of 4000–400 cm^{-1}.

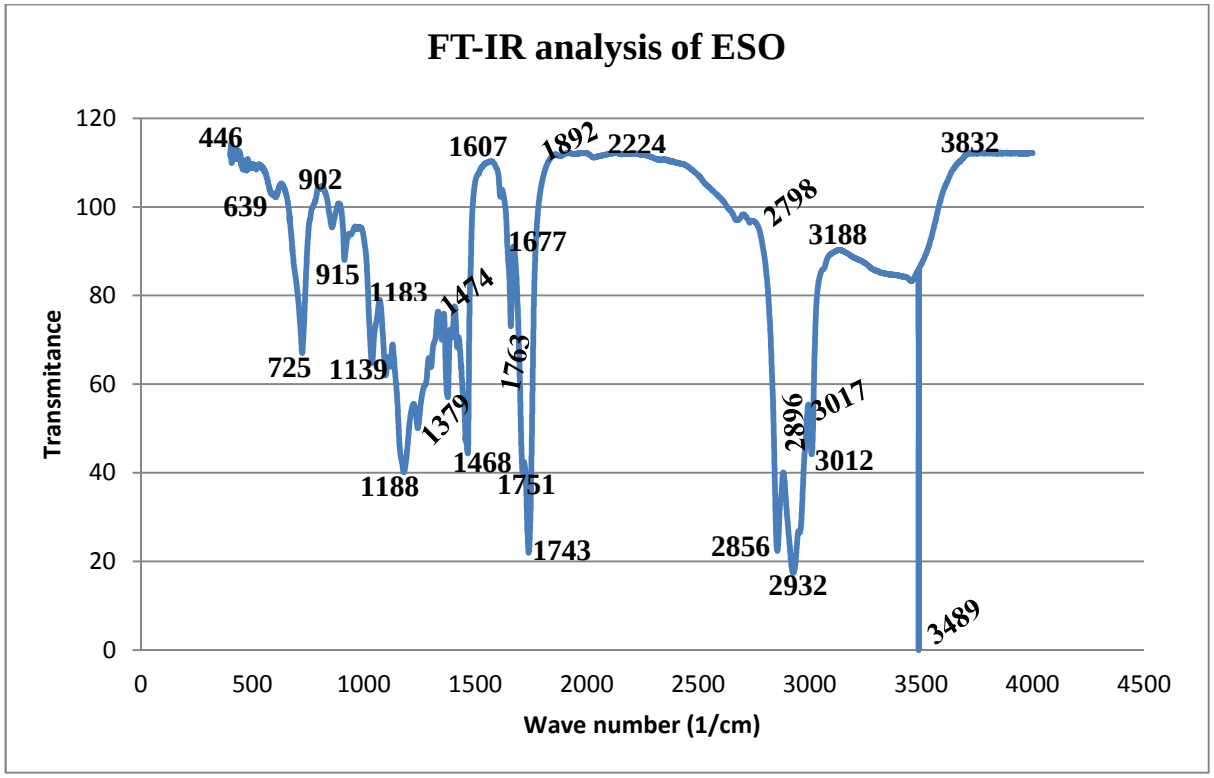


Figure 4-8: FT-IR analysis of the extracted oil using Ethanol solvent

FTIR spectra of both oils appear similar. The results of FT-IR peak values and functional groups were represented in table 4.14. The result profile showed the presence of functional groups like amines (3489), alkanes (2935, 2856, and 1468), acids (1743, 1379), ester (1188), alky1 (1139), and alkenes (725 - 915) (K. Nivetha and G. Prasanna, 2016).

Table 4-13: Biological activities of the components

No	Name of compounds	CompoundNature	PA% HSO	PA % ESO	Activity
1	Tetradecanoic acid	Myristic acid		0.18	Antioxidant, Lubricant, Hypocholesterolemic, Cancer Preventive, Cosmetic
2	9,12-Octadecadienoicacid,(z,z)	Linoleic acid	23.11	10.39	Anti-inflammatory, Nematicide, Antihistaminic, Antiacne, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Ant arthritic, Antieczemic
3	9,12,15-Octadecatrienoic acid,	(z,z,z)-Linolenic acid	-	0.96	Anti-inflammatory, Insectifuge, Insectifuge, Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Antihistaminic, Antieczemic, Antiacne,
4	Hexadecanoic acid methyl ester	Fatty acid ester	12.94	0.14	Antioxidant, Flavor, Hypocholesterolemic pesticide, 5-Alpha reductase inhibitor
	Hexadecanoic acid, ethyl ester	Stearic acid	-	11.31	Cancer preventive, Insectifuge.
5	Hexadecanoic acid	Palmitic acid	-	29.42	Antioxidant ,Hypocholesterolemic Nematicide, pesticide, Lubricant, Ant androgenic, Flavor, Hemolytic 5- Alpha reductase inhibitor
6	Thymoquinone	(2-isopropyl-5 methylbenzoquin	-	0.03	Anticancer activity
7	Thymol	Biocides	2.88	0.06	Antimicrobial activity, Antitumor properties.
8	Oleic Acid	Free Fatty acid	-	0.65	Monoacylglycerol, Antioxidant, antiatheroselerotic and protein glycation inhibitory activities.
9	Palmitic acid	Nematicide	2.28	2.92	Antioxidant

Table 4-14: FT-IR analysis of the extracted oil

No.	Peak value	Bond	Functional group
1	3489	NH-Stretch	Amine
2	2935	C-H Stretch	Alkane
3	2856	C-H Stretch	Alkane
4	1743	C = O Stretch	Acid
5	1468	C-H bending	Alkane
6	1379	C – O Stretch	Acid
7	1188	C – O Stretch	Ester
8	1139	C – F Stretch	Alkyl
9	915	=C-H bending	Alkene
10	725	=C-H bending	Alkene

4.5.5. Physical and Chemical analysis of the refined Fixed oil

Using the various formulae as indicated in the experimental procedure and doing the analysis of the crude oil, physical and chemical properties of the virgin (unrefined) calculated and finally summarizes the results and presented in Table 4.15 and Table 4.16.

Table 4-15 : Physical properties of the crude and refined black cumin oil

Property	**Crude black HSO**	**Refined HSO**	**Crude black ESO**	**Refined black ESO**
Specific gravity	0.9199	0.9196	0.9215	0.9158
Viscosity (m^2/s)	$7.015*10^{-2}$	$5.925*10^{-2}$	$7.298*10^{-2}$	$6.382*10^{-2}$
pH	6.28	6.37	5.98	6.35

Table 4-16: Chemical properties of the crude and refined black cumin oil

Property	**Crude HSO**	**Refined HSO**	**Crude ESO**	**Refined ESO**
Acid Value (mg NaOH/g of oil)	0.068	0.064	0.048	0.042
Saponification Value (mg KOH/g of oil)	202.01	196.52	197.62	193.68
Iodine Value (g I_2/100g of oil)	91.45	84.42	74.346	70.83

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusions

Black cumin seeds are rich sources of oils containing diverse group of phytochemicals. The presence of these bioactive compounds justifies the use of the seeds for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results.

This study intended with the extraction of Black cumin seed oil using soxhlet extraction and used two solvent separately named as ethanol and hexane solvents. The result would be analyzed and optimized by using two factors; seed to solvent ratio (with levels of 1 to 4; 1 to 6 and 1 to 8) and extraction time (with level of 2hr, 4hr, 6hr and 8hr). The maximum results that obtained from this study would be 35.725% using hexane solvent with seed to solvent ratio of 1:8 and 4 hour of extraction and 35.51% using Ethanol solvent with seed solvent ratio of 1:6 and extraction time of 6 hour. The minimum oil yield that we got from this thesis would be when the seed to solvent ratio and the extraction time decreases.

From design expert software the analysis of variables (ANOVA) with P value < 0.0001 for seed to solvent and extraction time with black cumin oil using hexane and ethanol indicate that the operating parameters have significant effect on oil yield. But the interaction of the two factors (that was AB) is not significant because the p value is > 0.05 that was 0.293 and 0.8143 in hexane and ethanol solvent respectively.

Gas Chromatography – Mass Spectroscopy (GC-MS) analysis of the extracted oil revealed the presence of well-known chemical compound in the sample. Using these machine 23 compounds was identified. The maximum yield in percent would be 12.94% Palmitic acid, 23.11% Linoleic acid and 25.27% of 6-Octadecenoic acid, methyl ester, (z)-. Those compounds may employ as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicidal activities. The fatty acid composition of the extracted oil with major components, and for the determination of functional group we used FTIR. In both solvents extracted oils, they have the same functional groups like amines, alkanes, acids, ester, alky1, and alkenes.

The physical and chemical properties of the extracted oil before and after refined had been measured and the physical properties such as the specific gravity, pH, and viscosity of HSO before refined would be 0.9199, 6.28, and 7.015 * 10^{-2} m^2/s respectively and after refined the result would be 0.916, 6.37, and 5.925 * 10^{-2} m^2/s respectively. Whereas for ESO before

refined the specific gravity, pH, and viscosity would be 0.9215, 5.98 and 7.298 * 10^{-2} m^2/s respectively and after refined the result would be 0.9158, 6.35 and 6.38 * 10^{-2} m^2/s respectively. The result indicated that the ESO is higher specific gravity and viscous than the HSO and in pH value both of the extracted oil has almost the same.

The chemical properties such as Acid value, saponification value and Iodine value also determined for both solvent extracted oil before and after refining. So, the Acid value(in mg NaOH/g of oil), Saponification value (mg KOH/g of oil) and Iodine value(g I_2/100g of oil) for HSO before refining would be 0.068, 202.01 and 91.45 and for the refined oil 0.064, 196.52 and 84.42 91.45 respectively. In similar way for ESO the Acid value (in mg NaOH/g of oil), Saponification value (mg KOH/g of oil) and Iodine value (g I_2/100g of oil) of crude oils would be 0.048, 197.62 and 74.346 and for refined ESO 0.042, 193.68 and 70.83.

As the result shows ESO is low saponification value, acid value and iodine value than HSO. Lower acid value means that the oil samples contain less free fatty acids thus reducing its exposure to rancidification. Therefore HSO is quickly rancid than ESO. The saponification value of ESO would be lower than HSO these means the lower saponification value might be indicated that the level of impurities of ESO is higher than that of HSO and the Iodine value of ESO would be less than HSO. The iodine value or iodine number is the generally accepted parameter expressing the degree of unsaturation in the oil. So the higher iodine value, the greater the degree of unsaturation. Therefore, the degree of unsaturation of ESO would be less than that of HSO.

Finally we conclude that extraction of fixed oil solvent from black cumin seed is the most promising technology process and getting the maximum yield. According to the result there is no any insignificant effect while we extracting the fixed oil using ethanol solvent. All the physical and chemical properties of the oil results are not extremely higher or lower than the standards but a trace of hexane had be contain on the oil when we were using hexane solvent for extraction. But on ethanol solvent extraction if there is a trace of ethanol in the oil, there is no any significant effect on the human health and environments. So it is better to use Ethanol rather than hexane to produce the fixed oil when we are using solvent extraction process.

5.2. Recommendations

Based on the current investigation the following recommendations are forwarded:

- Further researches have to be carried out to increase the yield of the black cumin seed fixed oil by using other optimization parameter rather than seed to solvent ratio and extraction time, such as particle size, temperature and pressure.
- Soxhlet extraction is not feasible for commercial purpose. So further researchers have to be used other extraction process by controlling the optimum parameters.
- Detailed economic feasibility studies in the extraction process for producing this important oil in our country recommended, since it is critical for the rational of commercialization rather than importing the oil from abroad
- Further researches have to do other analysis and process of the oil for preparing for pharmaceutical use and food additives.

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APPENDICES

Appendix A

- Formulas and Equations used for characterization of the oil

Appendix B

- Experimental design analysis of the laboratory result

Appendix C

- GC – MS analysis of the sample

Appendix D

- Image of Laboratory equipments and experimental process

Appendix A

Formulas and Equations used for characterization of the oil

1. percentage of extracted oil $X\frac{\text{mass of oil (in gm)}}{\text{mass of sample (in gm)}}*100\%$
2. specific gravity $X\frac{\text{density of the oil}}{\text{density of the water}}$
3. kinematic viscosity of the oil(V) $X\frac{\text{«}}{\text{°}}$

   Where: Where μ = Dynamic Viscosity and

   ρ = Density of oil
4. Acid value (AV) $X56.1*\frac{N*V}{W}$

   Where: Where V = volume of standard potassium hydroxide (ml),

   N = Normality of potassium hydroxide solution

   W= sample weight (in g)
5. FFA as oleic acid $X28.2*\frac{N*V}{W}$ percent by weight

   Where V = volume of standard potassium hydroxide (ml),

   N = Normality of potassium hydroxide solution

   W= sample weight (in g)
6. Saponifica tion value (S.V) $X56.1*\frac{N*(B-S)}{W}$

   Where B = the volume in ml of standard HCl required for blank test;

   S = the volume in ml of standard HCl required for the Sample;

   N = Actual normality of the HCl used;

   W = Weight of oil taken in gram.
7. Iodine value (I.V) $X12.69*\frac{N*(B-S)}{W}$

   Where N = Normality of standard sodium thiosulphate used;

   B = Volume in ml of sodium thiosulphate used for blank;

   S= Volume in ml of sodium thiosulphate used for determination,

   W = Mass of the sample.

Appendix B

B. Experimental design analysis of the laboratory result

B_1: Design summery

Std	Run	Block	Factor 1 A: seed:solvent (m/v)	Factor 2 B: time(hr)	Response 1 Yield using Hexane solvent	Response 2 Yield using Ethanol solvnet
16	1	Block 1	6	6	7.139	6.906
20	2	Block 1	4	8	5.163	5.218
14	3	Block 1	4	6	4.795	5.023
24	4	Block 1	8	8	7.121	6.903
19	5	Block 1	4	8	4.682	4.994
5	6	Block 1	8	2	5.846	6.255
22	7	Block 1	6	8	6.623	6.975
1	8	Block 1	4	2	3.938	4.275
12	9	Block 1	8	4	7.536	6.835
2	10	Block 1	4	2	4.292	4.849
18	11	Block 1	8	6	7.358	6.925
15	12	Block 1	6	6	6.615	7.298
10	13	Block 1	6	4	6.236	6.291
3	14	Block 1	6	2	5.181	6.072
8	15	Block 1	4	4	4.501	4.698
9	16	Block 1	6	4	6.824	6.521
6	17	Block 1	8	2	5.172	6.387
4	18	Block 1	6	2	4.824	6.238
17	19	Block 1	8	6	6.735	6.964
13	20	Block 1	4	6	5.049	5.251
21	21	Block 1	6	8	7.141	6.511
11	22	Block 1	8	4	6.754	6.533
7	23	Block 1	4	4	5.091	5.144
23	24	Block 1	8	8	6.924	6.637

Where: for seed to solvent ratio

4 = 1 to 4 ratio
6 = 1 to 6 ratio
8 = 1 to 8 ratio

B_2: Fit summery of the yield

For Hexane solvent

Sequential Model Sum of Squares

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	834.73	1	834.73			
Linear vs Mean	26.46	5	5.29	36.45	< 0.0001	Suggested
2FI vs Linear	0.97	6	0.16	1.19	0.3747	
Residual	1.64	12	0.14			
Total	863.80	24	35.99			

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	5.95	9	0.66	4.84	0.0067	
2FI	5.77	8	0.72	5.28	0.0053	
Quadratic	1.11	6	0.19	1.36	0.3073	Suggested
Cubic	0.14	3	0.047	0.35	0.7927	Aliased
Pure Error	1.64	12	0.14			

"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

ForEthanol solvent

Sequential Model Sum of Squares

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	884.56	1	884.56			
Linear vs Mean	17.29	5	3.46	78.42	< 0.0001	Suggested
2FI vs Linear	0.16	6	0.027	0.52	0.7841	
Residual	0.63	12	0.053			
Total	902.64	24	37.61			

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	4.06	9	0.45	8.59	0.0005	
2FI	4.06	8	0.51	9.65	0.0003	
Quadratic	0.29	6	0.048	0.91	0.5222	Suggested
Cubic	0.12	3	0.040	0.76	0.5395	Aliased
Pure Error	0.63	12	0.053			

B_3: Analysis of variance table for extraction yield

Analysis of variance table for extraction yield using Hexane solvent

Source	Sum of squares	Degree of Freedom	Mean square	F- value	P-value
Model	26.32	5	5.26	**34.46**	**0.0001**
A - Seed: solvent	15.87	1	15.87	103.89	**0.0001**
B – Time	5.61	1	5.61	36.74	**0.0001**
AB	0.18	1	0.18	1.17	**0.2930**
A^2	2.17	1	2.17	14.21	**0.0014**
B^2	2.49	1	2.49	16.28	**0.0008**
Residuals	2.75	18	0.15		
Lack of Fit	1.11	6	0.19	1.36	**0.3073**
Pure Error	1.64	12	0.14		
Total	29.07	23			

Analysis of variance table for extraction using Ethanol solvent

Source	Sum of squares	Degree of Freedom	Mean square	F- value	P-value
Model	17.17	5	3.43	**67.48**	**0.0001**
A - Seed: solvent	12.23	1	12.23	240.30	**0.0001**
B – Time	1.17	1	1.17	22.92	**0.0001**
AB	2.892E-003	1	2.892E-003	0.057	**0.8143**
A^2	3.38	1	3.38	66.38	**0.0001**
B^2	0.39	1	0.39	7.74	**0.0123**
Residuals	0.92	18	0.051		
Lack of Fit	0.29	6	0.048	0.91	**0.5222**
Pure Error	0.63	12	0.053		
Total	18.08	23			

B_4: Development of Model equation

Model summery test

Model Summary Statistics for Hexane solvent

Source	Std. Dev	R-Squared	Adjusted R-Square	PredictedR-Squared	PRESS	
Linear	0.60	0.7390	0.7141	0.6634	9.79	
2FI	0.61	0.7452	0.7069	0.6252	10.89	
Quadratic	**0.39**	**0.9054**	**0.8791**	**0.8348**	**4.80**	**Suggested**
Cubic	0.34	0.9387	0.9061	0.8451	4.50	**Aliased**

Model summery statistics for Ethanol solvent

Source	Std. Dev	R-Squared	Adjusted R-Square	**PredictedR-Squared**	**PRESS**	
Linear	0.47	0.7406	0.7159	0.6732	5.91	
2FI	0.48	0.7408	0.7019	0.6328	6.64	
Quadratic	**0.23**	**0.9494**	**0.9353**	**0.9101**	**1.63**	**Suggested**
Cubic	0.22	0.9585	0.9364	0.8909	1.97	**Aliased**

Development of regression model equation

Equation in terms of Coded factor

For hexane solvent

yield using hexane solvent = +6.73 + 1.00 * A + 0.65 * B +0.14 * A * B - 0.64 *A^2 – 0.72 *B^2

For Ethanol solvent

yield using ethanol solvent = +6.76 + 0.87 * A + 0.30 * B - 0.018 * A * B - 0.80 * A^2 - 0.29 * B^2

Where A= Seed solvent ratio

B= time

Equation in terms of Actual factor

For hexane solvent

yield using hexane solvent = - 4.38835 + 2.29381 * seed:solvent +0.87905 * time +0.023669 * seed:solvent * time - 0.15952 * seed:solvent^2 -0.080479 * time^2

For ethanol solvent

yield using ethanol solvent= - 4.40715 + 2.83956 * seed:solvent + 0.43694 * time - 3.00625E-003 * seed:solvent * time - 0.19895 * seed:solvent^2 - 0.032031* time^2

B_5: Model Adequacy checking

Violation of the basic assumptions and model adequacy can be easily investigated by the examination of Residuals. And first we see the model adequacy measure that described in the table below.

Model adequacy measures using hexane solvent

Std. Dev.	0.39	R-Squared	0.9054
Mean	5.90	Adj R-Squared	0.8791
C.V. %	6.63	Pred R-Squared	0.8348
PRESS	4.80	Adeq Precision	17.429

Model adequacy measures using Ethanol solvent

Std. Dev.	0.23	R-Squared	0.9494
Mean	6.07	Adj R-Squared	0.9353
C.V. %	3.72	Pred R-Squared	0.9101
PRESS	1.63	Adeq Precision	21.377

The "Pred R-Squared" of 0.8348 is in reasonable agreement with the "Adj R-Squared" of 0.8791 for hexane solvent. Similarly for ethanol solvent from the table 4.9 shows The "Pred R-Squared" of 0.9494 is in reasonable agreement with the "Adj R-Squared" of 0.9353.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 17.429 indicates an adequate signal. The same principle used for the ethanol solvent yield model the ratio of 21.377 indicates an adequate signal.similarly This model can be used to navigate the design space.

B_6: Normality Assumption on extraction yield

Figures 1 to Figures 2 shows how exactly the produced oil yield is modeled. The points line up well and the deviation of points for oil yield from normality is insignificant. In addition, the normal probability plot indicates the residuals following a normal distribution. In the case of this experiment the points in the plots shows fit to a straight line, this shows that the quadratic polynomial model satisfies the assumptions of analysis of variance (ANOVA) i.e. the error distribution is approximately normal. Ideally the normal plot will be a straight line, indicating no abnormalities. There is no severe indication of abnormality, nor is there any evidence pointing to possible outliers. The points are coded by color to the level of response and they represent going from cool blue for lowest values to hot red for the highest. Only the studentized form of residuals will produce valid diagnostic graphs.

As we can see in both figures the graph Look only for definite patterns like an "S-shaped" curve, which indicates that a transformation of the response may provide a better analysis and The tendency of the normal probability plot to bend down slightly on the left side and upward slightly on the right side implies that the tails of the error distribution are somewhat thinner than would be anticipated in a normal distribution; that is, the largest residuals are not quite as large (in absolute value) as expected. The general impression from examining the display is that the error distribution is approximately normal.

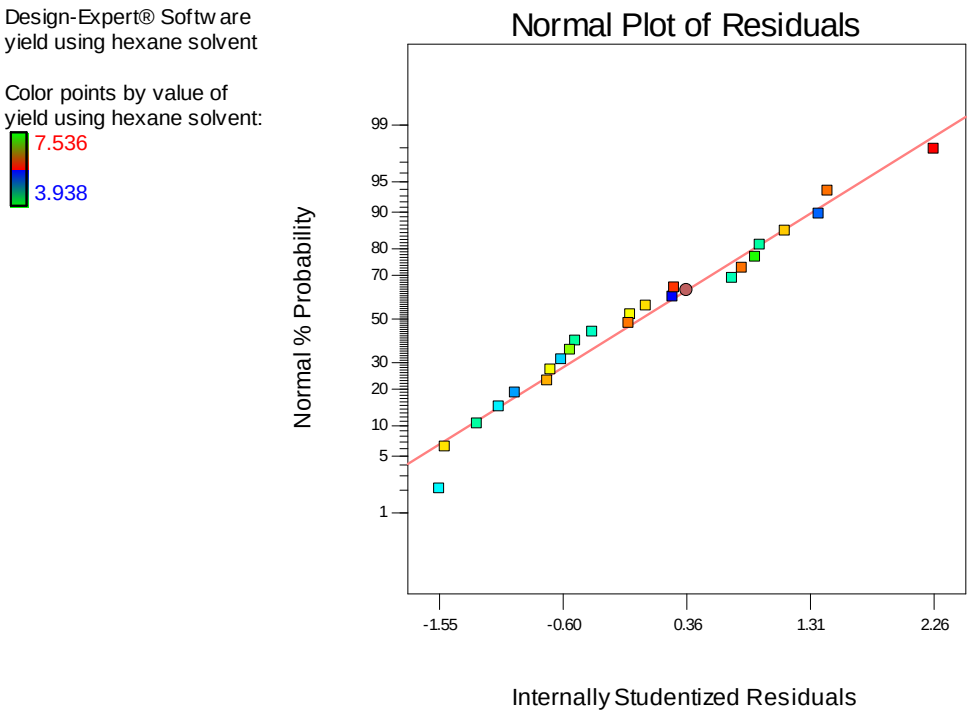


Figure 1: Normal probability plot of residuals for hexane solvent extraction

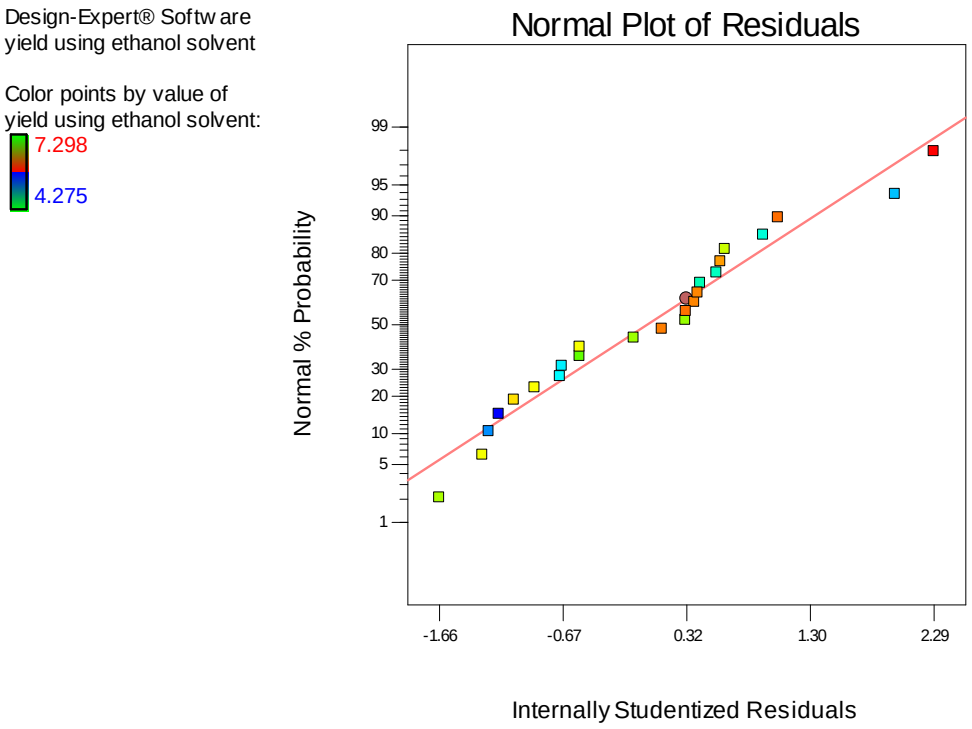


Figure 2: Normal probability plot of residuals for ethanol solvent extraction

Plot of Residuals versus Fitted Values

The assumptions of the analysis of variance (ANOVA) should be checked using the plots of residuals versus predicted. This could be useful to know how much the model is acceptable. In the analysis of variance, it is usually more effective to do this with the residuals. When the model is correct and the assumptions are satisfied, the residuals is structure less; in particular, they are unrelated to any other variable including the predicted response. A simple check is to plot the residuals versus the fitted, predicted values of the model.

Figure 5 and 6 shows that the plots of the residuals versus the fitted va1ue for hexane solvent extraction and ethanol solvent extraction there is no unusual structure is apparent on the graph, it is structureless. The plot shows random scatter which justifying no need for an alteration to minimize personal error. There for the model is correct and the assumption that we take for the model is satisfied.

Predicted versus actual value

It is the graph of the actual response values versus the predicted response values which helps to detect a value, or group of values, that are not easily predicted by the model. It is also important to check whether the regression model is fairly well fitted with the observed values, by comparing each observed value with predicted value Figure 3 and 4 shows that how the predicted value and actual values of each run approach the straight line. The straight line shows the predicted and actual values are closer to each other. When the point is above the straight line predicted value is greater than the actual value and the reverse is also true. That means when the actual value is less than the predicted value the point lies below the straight line. The other condition is when the point lies on the straight line that run has the same actual and predicted value. To sum up, in both figures 4.5 and 4.6 the points laid on the straight line, so the actual and predicted values have closer value.

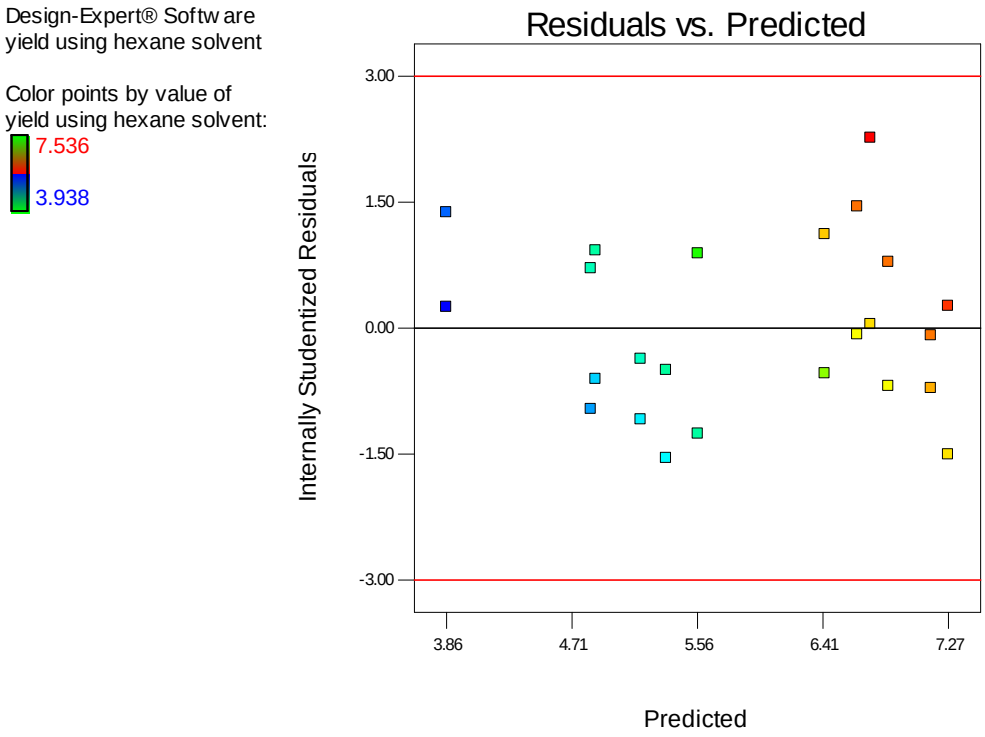


Figure 3: Plot of residuals versus time fitted values for hexane solvent extraction

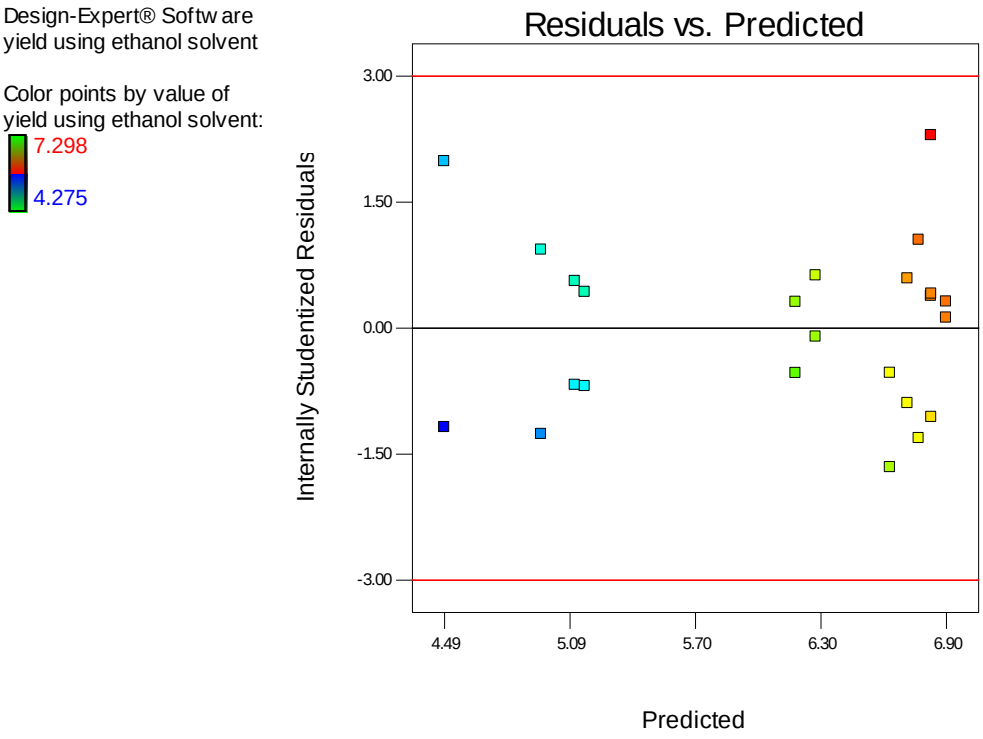


Figure 4: Plot of residuals versus time fitted values for ethanol solvent extraction

Plot of residuals versus time fitted values for ethanol solvent extraction

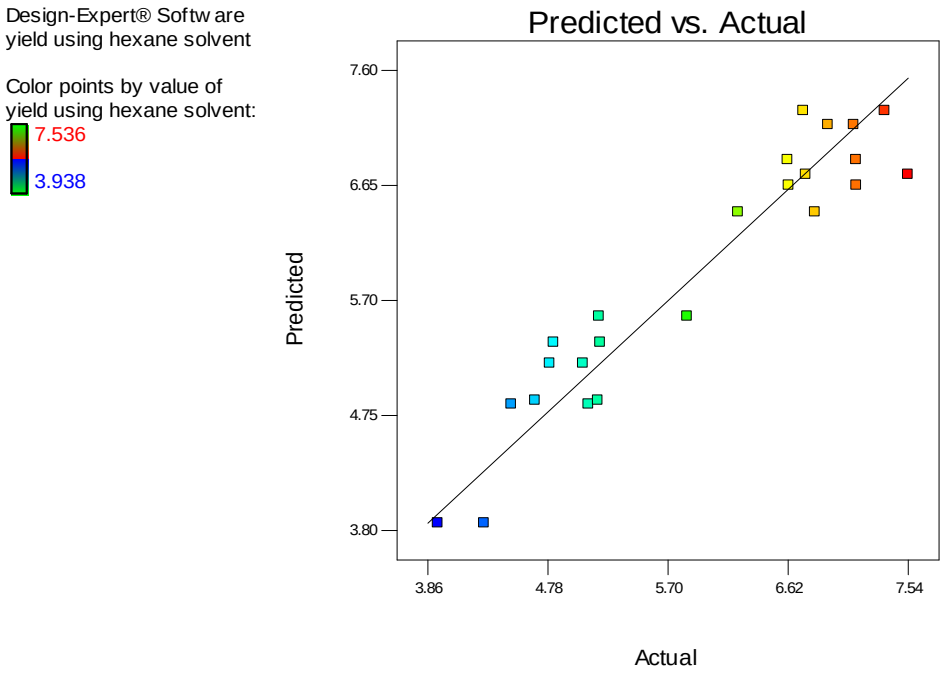


Figure 5: Predicted versus actual values of extraction yield on hexane solvent

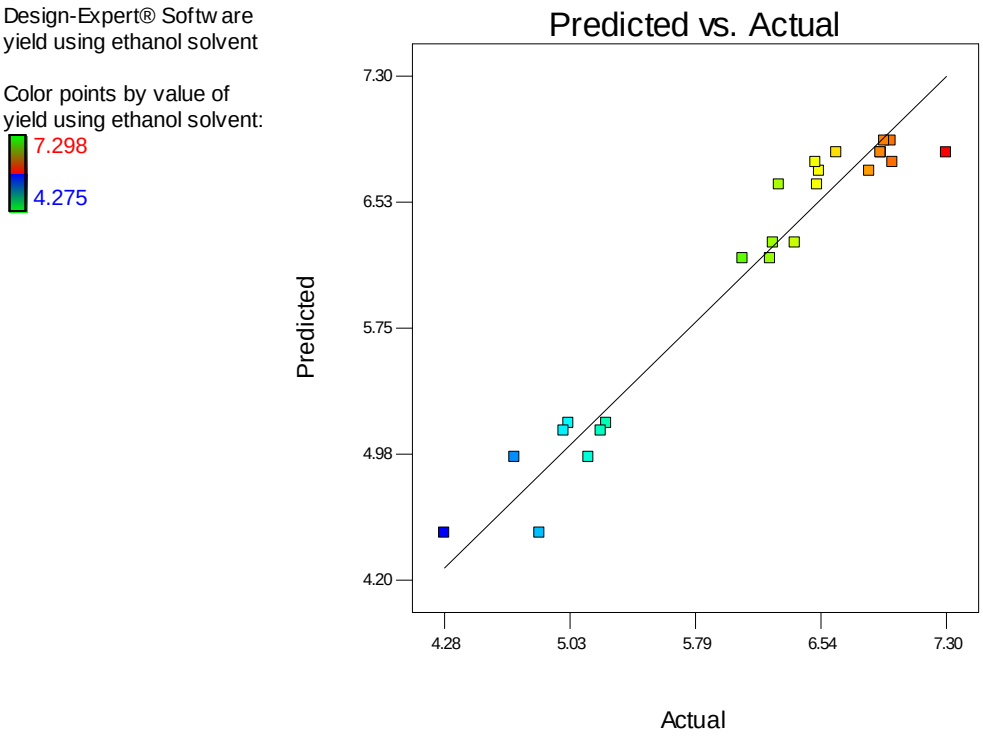


Figure 6: Predicted versus actual values of extraction yield on ethanol solvent

B_7: response surface diageram of the yield

For hexane solvent

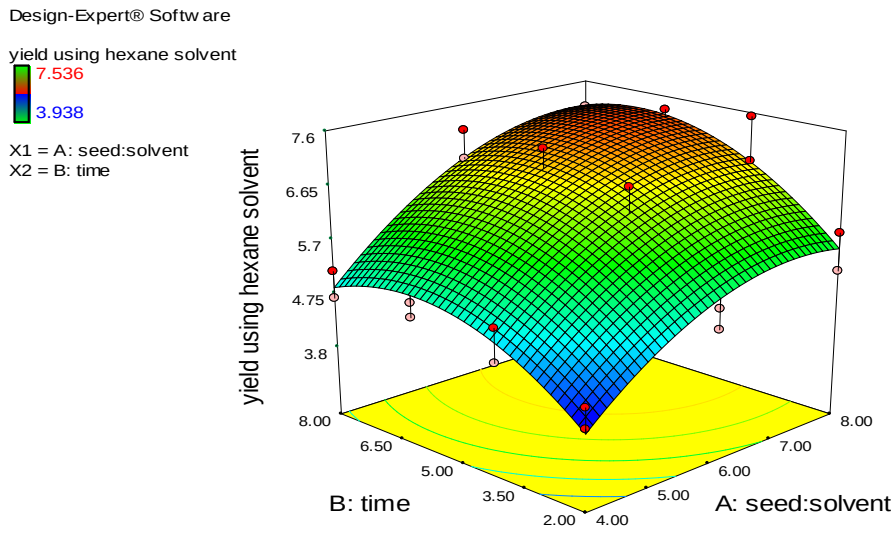


Figure 7: Response surface for hexane solvent

For ethanol solvent

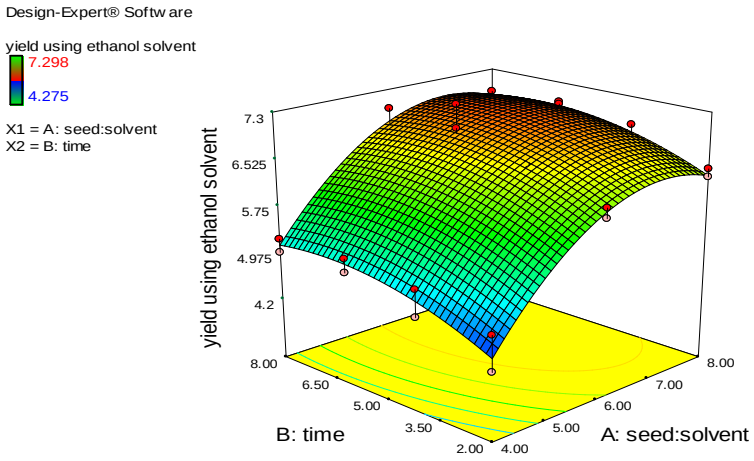
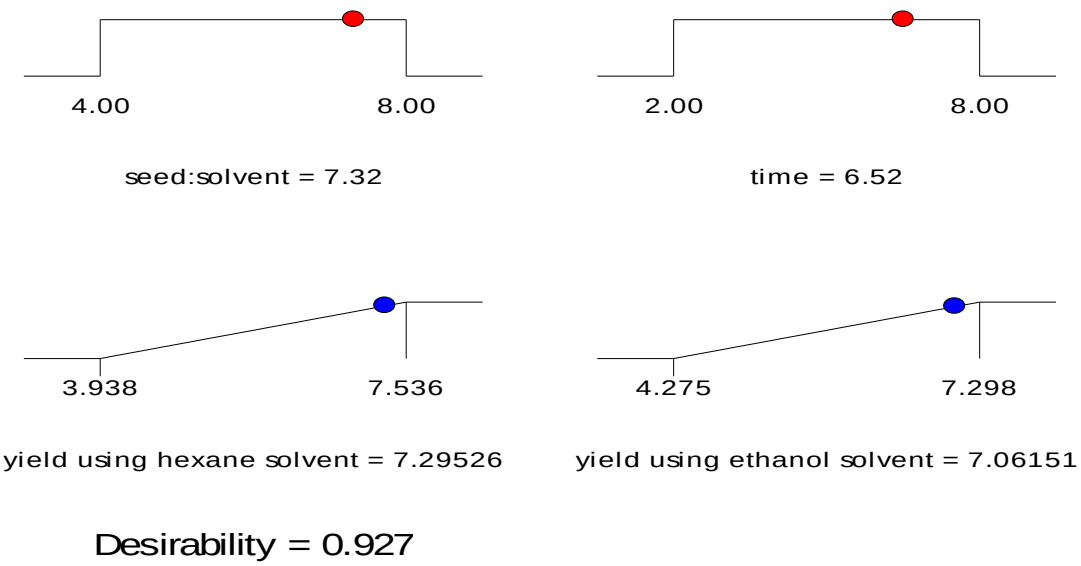


Figure 8: Response surface for ethanol solvent

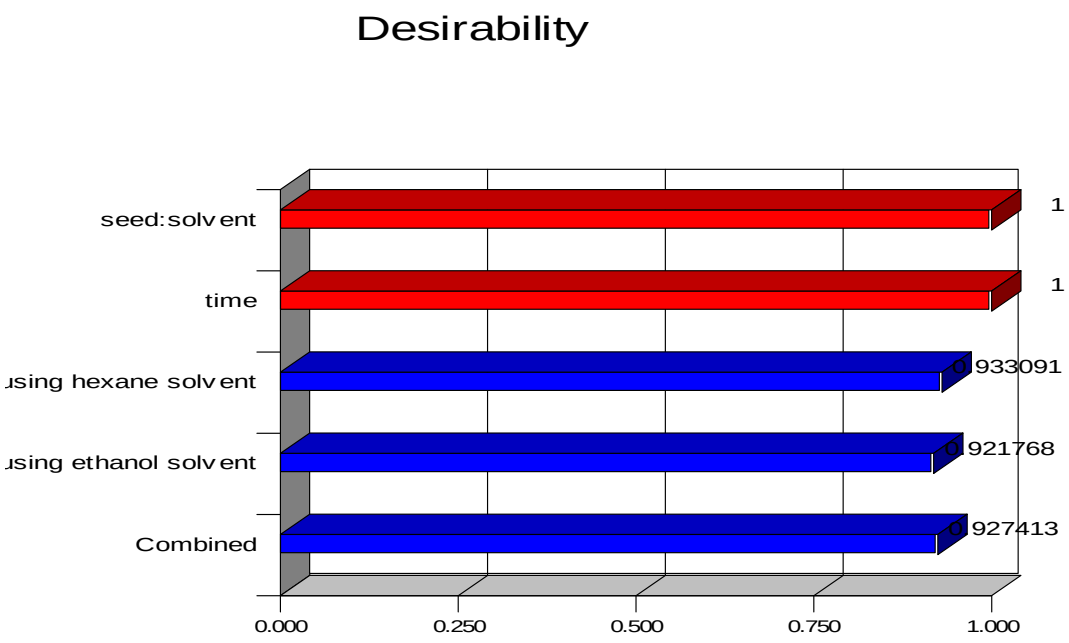
B_4 numerical optimizzation of the yield

Ramp function garph



Desirability = 0.927

Desirebility using Bar graph



Appendix C: Fatty acid components of the extracted oil

Appendix D: Image of laboratory equipements and experimental process
D1: raw material preparation

Black cumin seed

Dryinng by sun

Oven

Analytical Balance

D_2: lab equipements that used for extraction

Extraction thimble

Soxhlet extractor with condenser and water bath

Recovery process

Chiller

D_3: lab equipements that used for caracterization

Density measuring device **pH meter**

Visco meter **Cenrifuge**

D_4: Events when doing the experiment